

2 October 2009 | \$10

Science



Ardipithecus ramidus

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Ardipithecus ramidus

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COVER

Partial skeleton of *Ardipithecus ramidus*, a hominid species living about 4.4 million years ago in Ethiopia. This female stood about 1.2 meters high. Eleven papers from an international team of authors published in print and online in this special issue describe the anatomy of this species and its habitat and discuss the implications for understanding human evolution. One result is that extant great apes are poor models for our last common ancestor with chimpanzees. See page 60 for an introduction.

Image: © T. White, 2008

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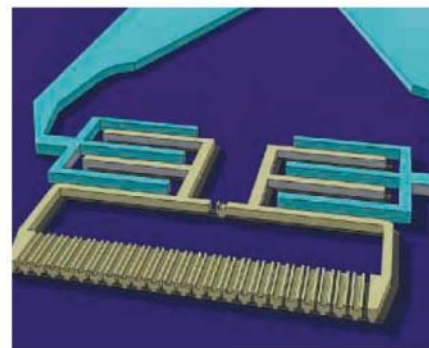
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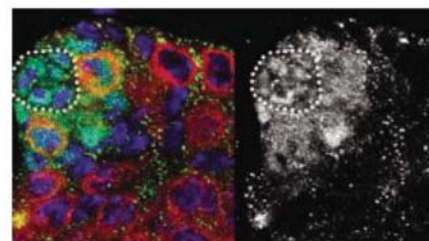
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SCIENCE SIGNALING

Responding to redox signals.



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SPECIAL FEATURE: *Ardipithecus ramidus*

The entire contents of this week's special section, online—plus special video and podcast features. Find it all at www.sciencemag.org/ardipithecus/.

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A History of Beginnings

SCIENCE INSIDER

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Science Policy News and Analysis

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ADVANCING SCIENCE, SERVING SOCIETY



<< Dog Coats Shed Genetic Secrets

The coats of domestic dogs show great variation—long, short, straight, wavy, curly, wiry, or smooth. To investigate how this variation arises, **Cadiou *et al.*** (p. 150, published online 27 August) performed genome-wide association studies on 80 different dog breeds. The coat phenotype could be dissected into three simple traits of length, curl, and growth pattern or texture with each trait controlled by one major gene, *FGF5* (fibroblast growth factor-5), *KRT71* (keratin-71), and *RSPO2* (R-spondin-2), respectively. In combination, variants in these three genes alone account for the vast majority of the coat phenotypes in purebred dogs in the United States. Thus, a small number of simply inherited traits can be remixed to create extraordinary phenotypic variation.

Ultimate Simulator

Many body problems are difficult to model analytically and are often so complex that they cannot be simulated accurately on a classical computer. Because quantum systems can be inherently correlated, it has been proposed that such systems could be used to simulate other complex problems. **Buluta and Nori** (p. 108) review the progress being made toward realizing quantum simulators, describing some of the implementations and potential applications of using such controlled quantum systems as simulator tools.

Quiet, Please

One approach for building quantum computers is based on superconductors with appropriately designed components to control the pairs of charges flowing through the circuits. However, at the single-electron level, required quantum noise—generated by quantum fluctuations and throwing offset charges into the device—presents a real problem in manipulating the delicate quantum states of the qubits. **Manucharyan *et al.*** (p. 113) present a clever piece of quantum circuit engineering that can suppress the effect of the quantum noise and allow the quantum circuit to operate without disturbance.

Unwelcome Dominance

Stratospheric ozone is depleted by many different chemicals; most prominently, chlorofluorocarbons (CFCs) responsible for causing the Antarctic ozone hole. Nitrous oxide is also an ozone-depleting substance that has natural sources in addition to anthropogenic ones. Moreover, unlike CFCs, its use and emission are not regulated by the Montreal Protocol, which has helped to reverse the rate of growth of the ozone

hole. Surprisingly, **Ravishankara *et al.*** (p. 123, published online 27 August; see the Perspective by **Wuebbles**) now show that nitrous oxide is the single greatest ozone-depleting substance that, if its emissions are not controlled, is expected to remain the dominant ozone-depleting substance throughout the 21st century. Reducing nitrous oxide emissions would thus enhance the rate of recovery of the ozone hole and reduce the anthropogenic forcing of climate.

Cleaning Solid Oxide Fuel Cells

Solid oxide fuel cells, which operate between 500° and 1000°C, transport oxygen through a ceramic material. At these temperatures, metals that catalyze hydrocarbon reforming reactions can also be incorporated so that conventional fuels such as methane can power the cell. One problem, however, has been rapid deactivation by sulfur impurities and carbon buildup. **Yang *et al.*** (p. 126; see the Perspective by **Selman**) report that doping of a barium zirconate-cerate with the rare-earths Y and Yb creates a material that transports both protons and oxygen ions at 750°C. This material, when used with nickel at the fuel cell anode, resists deactivation even when traces of hydrogen sulfide are present, apparently through enhanced ability to supply or remove water during surface reactions.

Stemming Stem Cell Displacement

Adult stem cell niches can contain multiple types of stem cells with coordinated regulation, but the mechanisms for these interactions are largely unknown. In the fruit fly testis, Janus kinase-

signal transducer and activator of transcription (JAK-STAT) signaling is needed for the maintenance of the resident germline and somatic stem cells. The signaling inhibitor SOCS36E is a known JAK-STAT target. **Issigonis *et al.*** (p. 153) now show that SOCS36E functions in the maintenance of the germline stem cell via suppression of JAK-STAT signaling, specifically in the somatic stem cells. This prevents the somatic stem cells from displacing neighboring germline stem cells in an integrin-dependent manner, allowing both stem cell populations to occupy the niche.

Nanotubes to Order

To exploit carbon nanotubes fully in electronic applications, one needs to be able to separate or synthesize either all semiconducting or all metallic tubes. However, unbiased synthesis conditions produce a mixture containing two-thirds semiconducting tubes and one-third metallic tubes. **Harutyunyan *et al.*** (p. 116) show that altering the carrier gas and temperature, in combination with oxidative and reductive species during the synthesis process modifies the catalyst particles during synthesis, which leads to the selective growth of metallic single-walled carbon nanotubes. Thus, the shape and morphology of the catalyst seeds can be tuned to grow carbon nanotubes of a specific chirality.



Algal Rebound

The extinction at the Cretaceous-Paleogene boundary 65.5 million years ago represented a sudden and dramatic disruption of global

Continued on page 15

ecosystems. **Sepúlveda et al.** (p. 129) now show, however, that algae recovered rapidly and that photosynthesis and primary production thus also recovered. The authors tracked algal productivity in the thick boundary layer in Denmark through a series of diagnostic biomarkers and isotopes. Algal productivity dropped abruptly during the extinction event but then recovered within the boundary layer, perhaps as quickly as within 50 years of the impact.

Extra Ancient Amber

Amber is fossilized tree resin, typically produced by trees in response to an injury. Most amber is Mesozoic or Cenozoic in age (dating back as far as 250 million years ago), and the most common class, produced primarily by angiosperms, is formed from distinct complex polyterpenoids. **Bray and Anderson** (p. 132; see the Perspective by **Grimaldi**) now find that amber from the Carboniferous, dating to more than 300 million years ago, long before the evolution of angiosperms, has a similar chemistry. Thus, the biosynthetic mechanism for producing complex ambers evolved long before the appearance of flowering plants.

Mosquito Vector Intervention

Mosquitoes are responsible for causing the infection of an estimated 120 million people with the nematode worms that block the lymph system and result in the gross pathology of elephantiasis and other filariases. **Kambris et al.** (p. 134) infected vector mosquitoes with a bacterium (*Wolbachia*), which impaired the insects' ability to act as filaria vectors and possibly would affect transmission of other pathogens, too. The infected mosquitoes were less susceptible to the worms owing to chronic up-regulation of mosquito immune responses. Immune activation bears a physiological cost for the mosquitoes, which may explain earlier observations of curtailed life spans of *Wolbachia*-infected mosquitoes.

Cultivating Farmers

Were the ancestors of modern Europeans the local hunter-gatherers who assimilated farming practices from neighboring cultures, or were they farmers who migrated from the Near East in the early Neolithic? By analyzing ancient hunter-gatherer skeletal DNA from 2300 to 13,400 B.C.E. **Bramanti et al.** (p. 137, published online 3 September) investigated the genetic relationship of European Ice Age hunter-gatherers, the first farmers of Europe, and modern Europeans. The results reject the hypothesis of direct continuity between hunter-gatherers and early farmers and between hunter-gatherers and modern Europeans. Major parts of central and northern Europe were colonized by incoming farmers 7500 years ago, who were not descended from the resident hunter-gatherers. Thus, migration rather than cultural diffusion was the driver of farming communities in Europe.



Mimicking Caloric Restriction

The extended life span and resistance to age-related diseases in animals exposed to caloric restriction has focused attention on the biochemical mechanisms that produce these effects. **Selman et al.** (p. 140; see the Perspective by **Kaeberlein and Kapahi**) explored the role of the mammalian ribosomal protein S6 kinase 1 (S6K1), which regulates protein translation and cellular energy metabolism. Female knockout mice lacking expression of S6K1 showed characteristics of animals exposed to caloric restriction, including improved health and increased longevity. The beneficial effects included reduced fat mass in spite of increased food intake. Thus, inhibition of signaling pathways activated by S6K1 might prove beneficial in protecting against age-related disease.

In and Out

For over 40 years, $\text{Ca}^{2+}/\text{H}^{+}$ antiporter has been reported across plasma cell membranes and mitochondrial inner membranes, but the molecules responsible for the exchange have not been known.

Jiang et al. (p. 144; see the Perspective by **Demaurex and Poburko**) conducted a genome-wide RNA interference screen in *Drosophila* and identified a nuclear-encoded mitochondrial protein, Letm1 (leucine zipper EF-hand-containing transmembrane protein 1), as a mitochondrial $\text{Ca}^{2+}/\text{H}^{+}$ antiporter critical for mitochondrial Ca^{2+} uptake. Furthermore, the gene's mammalian homolog is deleted in Wolf-Hirschhorn syndrome, a disorder characterized by mental retardation, microcephaly, seizures, hypotonia, and cleft lip or palate.

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Bruce Alberts is Editor-in-Chief of *Science*.

Understanding Human Origins

RESPONDING TO A QUESTION ABOUT HIS SOON-TO-BE-PUBLISHED *ON THE ORIGIN OF SPECIES*, Charles Darwin wrote in 1857 to Alfred Russel Wallace, “You ask whether I shall discuss ‘man’; I think I shall avoid the whole subject, as so surrounded with prejudices, though I freely admit that it is the highest and most interesting problem for the naturalist.” Only some 14 years later, in *The Descent of Man*, did Darwin address this “highest problem” head-on: There, he presciently remarked in his introduction that “It has often and confidently been asserted, that man’s origin can never be known: but . . . it is those who know little, and not those who know much, who so positively assert that this or that problem will never be solved by science.”

Darwin was certainly right. The intervening years provide conclusive evidence that it is very unwise to predict limits for what can be discovered through science. In fact, it now seems likely that, through synergistic advances in many disciplines, scientists will eventually decipher a substantial portion of the detailed evolutionary history of our own species at both the morphological and molecular levels.

First, what can we expect from paleoanthropology? In this 200th anniversary year of Darwin’s birth, *Science* is pleased to publish the results of many years of scientific research that suggest an unexpected form for our last common ancestor with chimpanzees. This issue contains 11 Research Articles involving more than 40 authors, plus News articles that describe the life and times of *Ardipithecus ramidus*, a hominid species that lived 4.4 million years ago in the Afar Rift region of northeastern Ethiopia. This region exposes a total depth of 300 meters of sediments that were deposited in rivers, lakes, and floodplains between about 5.5 and 3.8 million years ago. Even considering only this one site (there are many others), it is staggering to reflect on the huge number of hominid remains that can in principle be discovered, given sufficient time and effort. Moreover, the history of science assures us that powerful new techniques will be developed in the coming years to accelerate such research, as they have been in the past. We can thus be certain that scientists will eventually obtain a rather detailed record showing how the anatomy of the human body evolved over many millions of years.

What can we expect from a combination of genetics, genomics, biochemistry, and comparative organismal biology? We will want to interpret the history of the morphological transformations in the humanoid skeleton and musculature in terms of the molecular changes in the DNA that caused them. Genes and their regulatory regions control the morphology of animals through very complex biochemical processes that affect cell behavior during embryonic development. Nevertheless, experimental studies of model organisms such as fruit flies, worms, fish, and mice are advancing our understanding of the molecular mechanisms involved. New inexpensive methods for deciphering the complete genome sequence of any organism will soon accelerate this process, allowing scientists to analyze the recurring evolutionary morphological transformations that have been identified by organismal biologists,* so as to determine the specific DNA changes involved. And the DNA sequences that have changed most rapidly during recent human evolution are being cataloged, providing a new tool for finding important molecular differences that distinguish us from chimpanzees.†

The majesty of the discoveries already made represents a major triumph of the human intellect. And, as emphasized here, there will be many more discoveries to come. Darwin’s summary of his own efforts to understand human evolution is thus still relevant today: “Man may be excused for feeling some pride at having risen, though not through his own exertions, to the very summit of the organic scale; and the fact of his having thus risen, instead of having been aboriginally placed there, may give him hope for a still higher destiny in the distant future.”

— Bruce Alberts

10.1126/science.1182387



*R. L. Mueller et al., *Proc. Natl. Acad. Sci. U.S.A.* **101**, 3820 (2004). †S. Prabhakar et al., *Science* **314**, 786 (2006).

BIOTECHNOLOGY

Cutting Off Communication

Many bacteria communicate with one another through the secretion and uptake of small signaling molecules. If this process, known as quorum sensing, is disrupted, bacterial communities can no longer collectively respond to environmental stresses or cooperatively build complex structures such as biofilms. Yeon *et al.* have leveraged such a disruption to prevent biofouling—the clogging of membrane pores by accumulated particulate matter and bacteria—in wastewater treatment apparatus. The addition of a deactivating enzyme (acylase) quenched the quorum-sensing signal required for bacteria to form biofilms and thus increased the long-term performance of the filtration membrane. Furthermore, the use of a cell-sized magnetic carrier stabilized the acylase and facilitated its recovery and reuse over multiple operation cycles. Fortunately, the quenching process did not interfere with other beneficial bacterially driven processes in the bioreactor, such as the degradation of solid-phase organic matter. Although acylase is only specific to one type of signaling molecule, this procedure could eventually incorporate additional enzymes to quench the few other known quorum-sensing signals, effectively blocking all bacterial communication channels. — NW

Environ. Sci. Technol. **43**, 10.1021/es901323k (2009).

CHEMISTRY

Getting a Grip on Nitrogen

Chirality is associated more with carbon than with nitrogen centers, as the latter atoms tend to invert their configurations fairly rapidly on account of their unbonded electron pair. Recently, relatively slow nitrogen inversion was observed in cyclic oligomers of four or six α -amino acids (β -amino acids in which nitrogen replaces the traditional carbon at the β position) on account of hydrogen bonding among the substituents. Mocquet *et al.* now show that swapping in a single analogous residue that is chiral at carbon disrupts the collective inversion mechanism and thereby dramatically stabilizes the chiral nitrogen conformations throughout the ring. A variety of nuclear magnetic resonance and x-ray diffraction studies revealed a stable syndiotactic arrangement of asymmetric nitrogen centers up to temperatures of 413 K. — PDS

J. Am. Chem. Soc. **131**, 10.1021/ja9058074 (2009).

EVOLUTION

Background Matters

Species boundaries are often maintained because of reinforcement—where the male offspring of individuals of two different species are sterile. Although several candidate genes contributing to this reproductive barrier have

been identified, the mechanisms by which sterility is conferred are still generally unknown. The dominance theory suggests that sterility is caused by interactions of X-linked loci from one species with dominant autosomal loci in the other species. Chang and Noor have investigated this phenomenon by introgressing putative sterility quantitative trait loci (QTLs) from one fruit fly species into another, individually and in combination. They found that no single allele of any of the QTLs resulted in hybrid sterility but that sterility increased in the presence of other QTLs, even when all genes were heterozygous. These data suggest that the genetic background is responsible for modifying the degree of dominance and demonstrate that epistasis is an important component of hybrid male sterility. — LMZ

Evolution **63**, 10.1111/j.1558-5646.2009.00823.x (2009).

BIOPHYSICS

Packing It All In

Though the double helix structure of DNA has been known for half a century, it is only the starting point for arranging genetic material. Exactly how the 3 billion base pairs of human DNA, which are distributed unevenly across 23 chromosomes, are organized within a micron-sized nucleus, while retaining the flexibility to open and close individual regions so that spe-

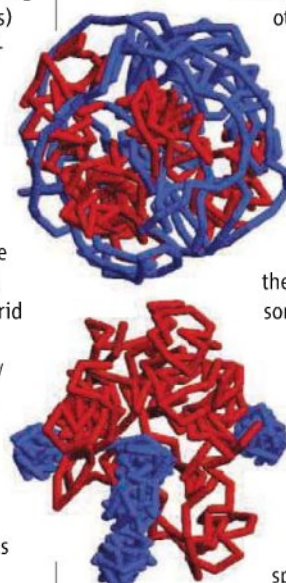
cific genes can be transcribed (which requires an unwinding of the double helix), is not entirely clear. The chromosomes are known to be spatially organized within the nucleus, in a function-dependent fashion; for instance, they each occupy distinct territories, and the general consensus is that in higher eukary-

otes gene-poor chromosomes are found preferentially at the periphery, with gene-rich chromosomes more centrally located.

Cook and Marenduzzo suggest that entropy may influence the positioning of chromosomes within nuclei. Using Monte Carlo simulation methods, they analyzed the distribution of stiff (blue) and flexible (red) self-avoiding polymers composed of strings of beads within a confined sphere, representing compact heterochromatin (blue)

and gene-rich chromatin (red) in the nucleus. The stiff polymers tended to localize to the periphery of the sphere, whereas the flexible ones moved to the center, analogous to the situ-

Continued on page 21



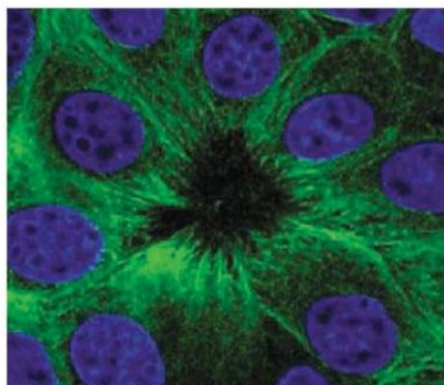
Continued from page 19

ation in vivo. Looping the polymers, which would represent the joining of distal regions of chromatin, promoted the formation of distinct territories, and flexible polymers were less likely to form contacts with other polymers, consistent with gene-rich regions being less frequently involved in chromosomal translocations. — HP
J. Cell Biol. **186**, 825 (2009).

CELL BIOLOGY

Squeezed Out by the Neighbors

Organs and tissues are surrounded by an epithelial layer of cells that serves as a physical barrier between, for instance, the blood and the vascular smooth muscle cells. If and when cells within



an epithelial layer die, they must be removed without breaching the integrity of the epithelial barrier. Slattum *et al.* have characterized the process by which apoptotic cells are actively extruded. There is a localized contraction of actin and myosin IIA within the cells (blue) that surround the dying cell—at their apical ends if the target cell is destined to exit into the tissue or, more commonly, at their basolateral surfaces if the cell is to be extruded into the luminal compartment. The latter requires microtubules (green) in the neighboring cells to reorient and to target a protein that controls actin and myosin activity, p115 RhoGEF, to the basolateral surface. In the whole organism, the direction of extrusion may figure in the subsequent fate of the ejected cell, particularly if, as may happen during tumorigenesis, the presumptive apoptotic cell does not in fact die. — SMH

J. Cell Biol. **186**, 693 (2009).

BIOMEDICINE

A Moving Target for Cancer Therapy

The primary cilium, an immotile structure found on many cells, is a critical site for regulating signaling by members of the Hedgehog family

of ligands. Hedgehog proteins have major roles in development, but inappropriate signaling through this pathway can contribute to certain cancers. Wong *et al.* and Han *et al.* have studied primary cilia in two cancers, human basal cell carcinomas and medulloblastomas. In keeping with earlier indications that events at the primary cilium can have both positive and negative effects on Hedgehog signaling, they found that the loss of cilia could either prevent or promote the growth of cancer cells, depending on how the Hedgehog pathway had been activated. If the cells expressed a constitutively active form of Smoothened, the upstream component that is activated when Hedgehog binds to the receptor Patched, ablation of the cilium inhibited tumor cell growth in mice. However, if the downstream transcription factor Gli2 was made constitutively active, the loss of cilia promoted tumor growth. The mechanisms at work are not precisely understood, but the authors discuss the implications for targeting therapies to inhibit Hedgehog signaling, where in some cases, the inhibition of ciliogenesis could exacerbate cancer growth rather than curb it. — LBR

Nat. Med. **15**, 1055; 1062 (2009).

ATMOSPHERIC SCIENCE

Accounting for Delay

Earth's early atmosphere was lacking in oxygen for nearly 2 billion years; a variety of data imply that the concentration of the oxidizing gas began to rise abruptly about 2.4 billion years ago. The degree to which biological processes may have contributed to this rise remains subject to debate—some evidence suggests that cyanobacteria, which produce oxygen as a by-product of photosynthesis, appeared at least several hundred million years earlier, but why then the delay? Two isotopic studies from rocks several hundred million years older than the prominent oxidation event imply that some feedbacks may have been involved. Godfrey and Falkowski show that nitrogen isotopes record a type of nitrogen cycling in the oceans requiring the presence of free oxygen. In turn, this cycling would have depleted inorganic nitrogen required by cyanobacteria or plankton for growth, limiting their oxygen production. Frei *et al.* report a chromium isotope record, which traces oxidative weathering. Their data implicate small and transient amounts of oxygen in the atmosphere and ocean during this time. Together these and other data imply that the final abrupt oxidation of Earth's atmosphere reflected some fits and starts over the previous several hundred million years. — BH

Nat. Geosci. **2**, 10.1038/ngeo633 (2009);

Nature **461**, 250 (2009).



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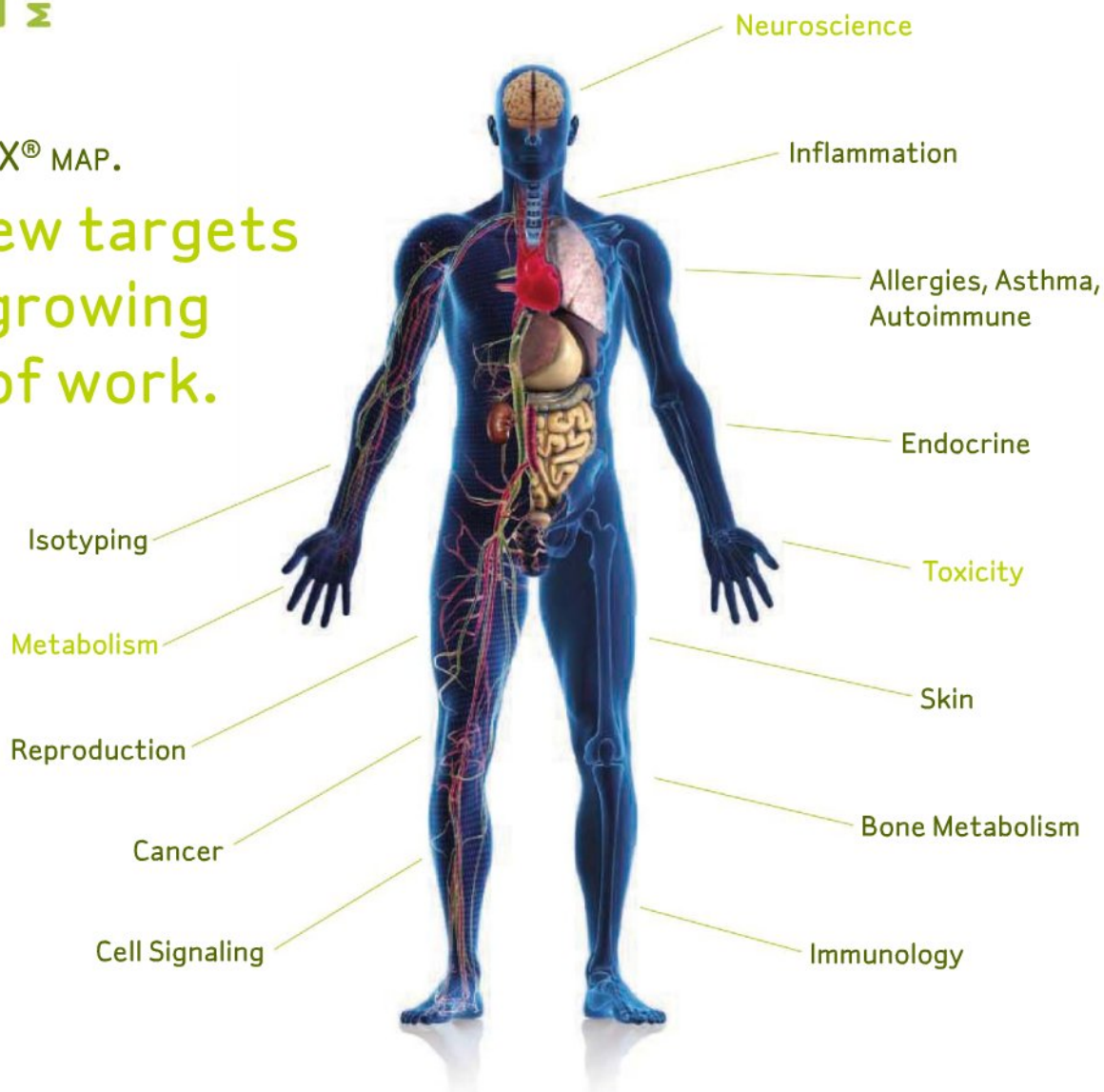
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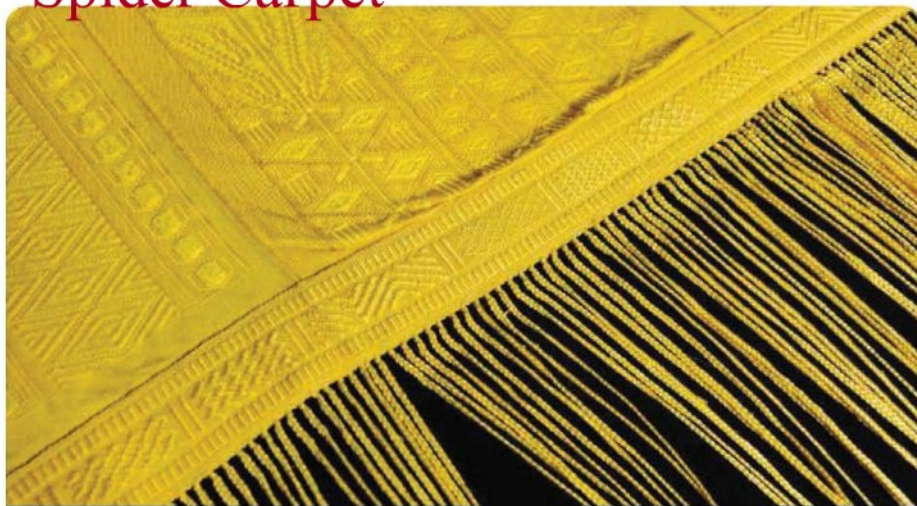
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Spider Carpet



A million female Madagascar golden orb spiders contributed their golden silk to this one-of-a-kind textile that went on display last month at the American Museum of Natural History in New York City. More than 80 people spent 4 years on the work, collecting spiders in the wild, drawing silk from immobilized (and later freed) arachnids with hand-powered machines, and twisting hundreds of spider lines to make each thread. The 3.4-by-1.2-meter tapestry is on loan from Simon Peers and Nicholas Godley, who founded Lamba, a weaving enterprise in Madagascar.

Shrinking the Shrinks

Many training programs for clinical psychologists in the United States should be scrapped, an organization of psychologists says. In a report to be released this month, the Association for Psychological Science (APS) calls for more scientific rigor in psychotherapy. "Clinical psychology resembles medicine at a point in its history when practitioners were operating in a largely prescientific manner," it says. Therapists' "lack of adequate science training ... leads them to value personal clinical experience over research evidence." The report lambastes the American Psychological Association (APA)—which comprises mainly clinical psychologists—for lax accreditation standards and proposes a new mechanism for certifying Ph.D. training programs.

Psychologist Scott Lilienfeld of Emory University in Atlanta praises the report, saying, "Far too many practitioners are administering unsubstantiated or untested intervention." But he worries that its proposals would freeze out Psy.D. programs, nonresearch degrees begun in the 1970s, which now turn out about half of the nation's clinical psychologists.

Jeffrey Zeig, a clinical psychologist and director of the Milton H. Erikson Foundation in Phoenix, says psychotherapy is much too diverse to be constrained by APS definitions. "There are more than 1,000,000 therapists in the U.S., and only a fraction" have Ph.D.s, says Zeig, who pre-

dicts the report "will have as much effect as a breeze has on a leaf."

But report co-author Timothy Baker of the University of Wisconsin School of Medicine and Public Health in Madison predicts that it "will ultimately reshape clinical psychology just as the [1910] Flexner Report reshaped medicine," leading to the closure of almost half the nation's medical schools.

Over the Top

Henry Hudson and others who searched for trade routes across the top of the world would be envious. Last month, with sea ice at its third-lowest extent ever, two German vessels made the first commercial transit all the way across the Arctic Ocean, carrying power plant components from South Korea to Novyy Port in Siberia and continuing on to Rotterdam, the Netherlands.



Bremen, Germany-based Beluga Shipping says it could have as many as six vessels making the trip in 2010, saving up to \$800,000 per trip. The route is only two-thirds as long as that through the Suez Canal.

With global warming, the Arctic "seems to be beckoning mariners," says Mead Treadwell, chair of the U.S. Arctic Research Commission. The Northern Sea Route skirting Russia and the fabled Northwest Passage over Canada have become increasingly navigable in recent summers, and traffic is growing. Treadwell says a 2004 study found more than 5000 vessels operating in or near the Arctic Ocean.

The melting sea ice is "an urgent wakeup call" about climate change, says Greenpeace spokesperson Melanie Duchin. Greenpeace has called for a moratorium on commercial activity to protect the area historically covered by sea ice year-round.

Mummy Recipe Hard to Follow

Frank Rühli wants to know just how the Egyptians did it. So he is trying to mummify human legs.

Rühli, a physician and head of the Swiss Mummy Project at the University of Zurich, and his collaborators severed the legs from a female donor body. One, the "control leg," was kept in an oven at 40°C and low humidity to replicate "natural mummification" in the Egyptian desert. The other leg, as described in ancient Egyptian records, was put on a pine board and covered with natron, a blend of four sodium compounds that pulls moisture out of the tissue. The researchers left it at 23°C to see what natron would do in the Swiss environment.

Other researchers have tried mummifying human remains. But the Swiss group is using advanced imaging technology, biopsies, and tests of DNA degradation for moment-by-moment analysis of the mummification process.

So far, the researchers have found that mummification in Zurich takes longer than expected: After 3 months, scans showed that the natron leg still had pockets of humidity, Rühli says. They have also discovered that storing an untreated leg in the heat doesn't work well. The control leg failed to dry out and started to decompose after a week. Rühli plans to repeat the experiment, this time encasing the control leg in hot sand.





U.K. nationality tests draw fire

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The origin of ecological structure

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Unexpected boost. Positive results from a massive AIDS vaccine trial in Thailand stunned the beleaguered field.



HIV/AIDS RESEARCH

Surprising AIDS Vaccine Success Praised and Pondered

Wow.

That was the reaction of many HIV/AIDS researchers last week to the news that a large clinical trial of an AIDS vaccine worked—the first success in 2 decades of effort. The two vaccines used in the study as a one-two punch had only a modest ability to protect people from HIV, and the results just barely qualified as statistically significant. Researchers will be trying to make sense of the somewhat baffling data for months if not years. But given all the failures in the field, many say that even this relatively weak signal is cause to celebrate.

The controversial \$105 million trial, the largest and most expensive AIDS vaccine study ever conducted, took 6 years and involved more than 16,000 volunteers at eight sites in Thailand. An equal number of people at average risk of becoming infected with HIV were randomly assigned to receive either the vaccine combo, tailor-made to protect against strains circulating in Thailand, or a saline placebo. Participants were mainly heterosexual, and about one-fourth reported high-risk behavior such as commercial sex work or intravenous drug use.

The trial was widely expected to fail because neither of the vaccines had done well

by themselves in clinical studies. Indeed, such lackluster results with one of the vaccines, a canarypox virus that has three HIV genes stitched into it, led the U.S. National Institute of Allergy and Infectious Diseases (NIAID) to pull the plug in 2002 on a large trial that mirrored the Thai study (*Science*, 1 March 2002, p. 1616). And the second vaccine, a recombinant form of the HIV surface protein gp120, later outright failed in two large-scale efficacy trials (*Science*, 21 November 2003, p. 1309). As a result, many investigators were dumbfounded to learn that at the end of the Thai study in June, 51 vaccinated people had become infected within 3 years of receiving their last shot, as compared with 74 people in the placebo group. The p value, which indicates whether results are due to chance, was less than 0.039, just below the widely accepted “significance” cutoff of 0.05. No serious adverse events were seen in either group.

Trial results were announced on 24 September in media briefings held by the U.S. Army, the main sponsor of the trial, and the Thai Ministry of Public Health. “Although the level of protection was modest, we think the study is a major scientific advance,” said Colonel Jerome Kim, HIV vaccines

product manager for the U.S. Army.

Even skeptics are careful not to rain on the parade. “Looking at the numbers, it’s underwhelming to me,” says Ronald Desrosiers, head of the New England Primate Research Center in Southborough, Massachusetts. “But I want to sit tight and get a bunch of people to do analyses and see whether the protective effect holds up.” Desrosiers was one of 22 researchers who argued in a *Science* Policy Forum that the study never should have been launched (*Science*, 16 January 2004, p. 316).

NIAID Director Anthony Fauci also has reservations. “This is a modest effect, and we have a lot of questions,” says Fauci, whose institute ended up funding about 75% of the study after Congress decided it should oversee the U.S. military’s AIDS program. Still, he is excited by the possibility that there’s even a trend toward efficacy. “If you look at the results of every other vaccine trial, it’s bupkis,” he said. “There isn’t even the hint of a signal.”

Supporters and critics alike agree that analyzing stored blood samples from the vaccinated people may uncover the immune responses that protected them. These so-called correlates of protection have been a Holy Grail to AIDS vaccine researchers, who have relied on different immunologic theories to design vaccines. “The real correlates of protection might be something we haven’t been examining up until this point,” says Norman Letvin, an AIDS vaccine researcher at the Beth Israel Deaconess Medical Center in Boston. “If that’s the case, it’s of enormous importance.”

It’s also possible that an AIDS vaccine may not have to be extremely powerful to work. “These vaccines induce some very weak immune responses, so in a sense this may be very good news because it may suggest that much less potent responses than we expected may have an impact on the acquisition of infection,” says Dennis Burton, an immunologist at the Scripps Research Institute in San Diego, California.

One of the most perplexing findings is that the vaccines prevented infection by HIV but failed to reduce levels of the virus in people who did become infected. “Most people expected it would be the other way around,” says Colonel Nelson Michael, director of the U.S. Military HIV Research Program.

That odd result in turn raises questions about the presumed “prime-boost” mecha-

CREDIT: U.S. MILITARY HIV RESEARCH PROGRAM



Ardipithecus
debuts

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nism of the vaccines. The canarypox vaccine, made by Sanofi Pasteur based in Lyon, France, primarily cranks up the cell-mediated arm of the immune system, which clears cells *after* they have become infected. The booster shot of gp120, supplied by Global Solutions for Infectious Diseases in South San Francisco, California, was supposed to somehow fortify that cell-mediated response. Even ardent proponents believed that this vaccine combo was more likely to blunt infections than prevent them. "Maybe we were wrong," says Michael.

"We finally have an argument that will be suffused with data rather than theories."

No one anticipates that these results will lead countries to license the vaccines, and even the trial's chief statistician acknowledges that a few more infected vaccinees could have tipped the scale toward statistical insignificance. "The results tell us two things: These vaccines work, and they don't work well enough," says statistician Donald Stablein, president of the Emmes Corp. in Rockville, Maryland.

Many researchers hope that when the full

data are released at an AIDS vaccine conference in Paris from 19 to 22 October, they will help with the design of future vaccines, as the information might lead to refined animal models and improved analyses of small-scale human studies. Skeptics, of course, will also be scouring the results to see whether factors such as risk behavior, circumcision, or the strains of the virus that caused infections might explain the odd results. "It's very early days," says Burton. "People should be enormously cautious now." **-JON COHEN**

SPACE SCIENCE

Mars Mission Delayed as Mad Dash to Prep Probe Falls Short

BEIJING—For the Russians, it would have been a bounce back from the disastrous launch of the Mars 96 spacecraft 13 years ago. For the Chinese, it would have been a maiden attempt to explore another planet. The two nations were gearing up to send a pair of probes next month to Mars and Phobos, the larger of the two martian moons, and return samples from Phobos—the first such mission in more than 30 years. But a breakneck effort to get the main spacecraft ready fell short, and last week the Russian Federal Space Agency, known as Roscosmos, announced that the launch will be postponed until 2011—the next favorable window.

"It's a pity," says Wu Ji, chief scientist for China's probe—Yinghuo-1—and director of the Center for Space Science and Applied Research here. The delay is unavoidable, adds Lev Zelenyi, director of the Space Research Institute (IKI) in Moscow. "We want to increase the reliability of the mission and test all systems again before final launch," he says. But there could be a silver lining: Russia may be able to add instruments to the payload, Zelenyi says.

Mars is a popular destination for planetary missions, with 39 having been launched to date. "Almost all focused on finding signs of life," says Wu. Russia's Mars 96 probe aimed to do that as well, and much more, but the spacecraft crashed after launch in November 1996, and Russia hasn't attempted a planetary mission since.

The Sino-Russian mission would offer a fresh approach. After a 10-month journey, the 11-ton Russian Phobos-Grunt spacecraft

would enter orbit and sling the bantamweight 110-kilogram Chinese probe, Yinghuo-1, or "firefly," into a 3-day elliptical orbit around Mars. Yinghuo-1 would take readings of the martian ionosphere and snap photos of the surface during low passes, hoping to catch the



Not going anywhere. A 2-year delay won't alter the science objectives at Phobos.

genesis of one of Mars's famous planet-sized dust storms. "Yinghuo-1 will be the first comprehensive survey of the martian space environment," says Wu. "We hope to do something that has never been done before." Phobos-Grunt, meanwhile, would transmit radio waves to Yinghuo-1 through the ionosphere to glean insights into its structure. After several months of studies in orbit, Phobos-Grunt would land on the martian moon, take soil samples, and head back to Earth.

Phobos-Grunt would also ferry the Liv-

ing Interplanetary Flight Experiment (LIFE): a titanium canister filled with 10 kinds of microbes from the three domains of life, bacteria, eukaryota, and archaea. The setup, provided by The Planetary Society in Pasadena, California, intends to test whether microbes—in this case a collection harmless to humans—can survive for years in space, mimicking conditions in which microbes might theoretically survive inside a meteoroid.

All that will have to wait. Rumors had been circulating for months that Phobos-Grunt might be postponed, but until last week the mission was a go, and Wu for one was planning to head to Kazakhstan for a late-October launch. His group's disappointment comes hard on the heels of news that China may not find the money to launch another mission, the Hard X-ray Modulation Telescope (*Science*, 25 September, p. 1613).

But more time might buy a better mission. In the next 2 years, IKI will undertake additional tests of its soil sampler. The delay should also allow them to restore to the payload a secondary ion spectrometer, a low-speed dust-particle detector, and perhaps an infrared spectrometer, says IKI's Alexander Zakharov, chief scientist for Phobos-Grunt. LIFE, meanwhile, is merely being put on hold. "We anticipate loading new organisms into a flight module in 2011," says LIFE's experiment manager, Bruce Betts. The Chinese and Russians insist they'll get the same data, just a tad later. After all, notes Zelenyi, "Phobos, as a celestial body, will not change in 2 years." **-RICHARD STONE**

CLIMATE CHANGE

What Happened to Global Warming? Scientists Say Just Wait a Bit

The blogosphere has been having a field day with global warming's apparent decade-long stagnation. Negotiators are working toward an international global warming agreement to be signed in Copenhagen in December, yet there hasn't been any warming for a decade. What's the point, bloggers ask?

Climate researchers are beginning to answer back in their preferred venue, the peer-reviewed literature. The pause in warming is real enough, but it's just temporary, they argue from their analyses. A natural swing in climate to the cool side has been holding greenhouse warming back, and such swings don't last forever. "In the end, global warming will prevail," says climate scientist Gavin Schmidt of NASA's Goddard Institute for Space Studies (GISS) in New York City.

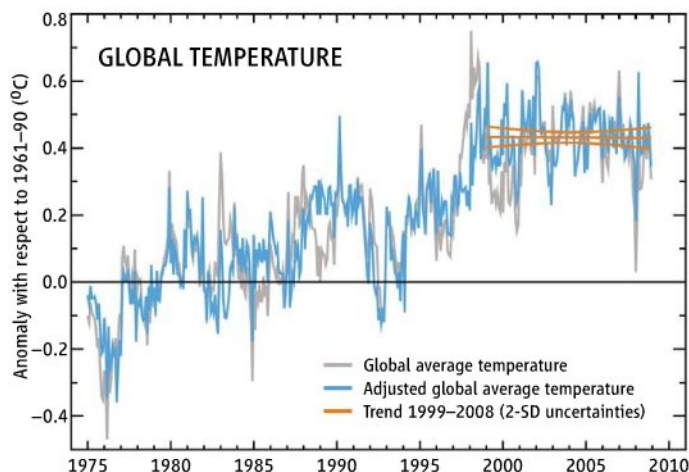
The latest response from the

climate community comes in *State of the Climate in 2008*, a special supplement to the current (August) issue of the *Bulletin of the American Meteorological Society*. Climate researcher Jeff Knight and eight colleagues at the Met Office Hadley Centre in Exeter, U.K.,

first establish that—at least in one leading temperature record—greenhouse warming has been stopped in its tracks for the past 10 years. In the HadCRUT3 temperature record, the world warmed by $0.07^{\circ}\text{C} \pm 0.07^{\circ}\text{C}$ from 1999 through 2008, not the 0.20°C expected by the Intergovernmental Panel on Climate Change. Corrected for the natural temperature effects of El Niño and its sister climate event La Niña, the decade's trend is a perfectly flat 0.00°C .

So contrarian bloggers are right: There's been no increase in greenhouse warming lately. That result came as no surprise to

Knight and his colleagues or, for that matter, to most climate scientists. But the Hadley Centre group took the next step, using climate modeling to try to quantify how unusual a 10-year warming pause might be. In 10 modeling runs of 21st century climate totaling 700 years worth of simulation, long-term warming proceeded about as expected: 2.0°C by the end of the century. But along the



Steady as she goes. The global average temperature (gray line; adjusted in blue) has remained steady the past 10 years, as shown by the orange trend lines.

BIOSECURITY

Lawmakers Signal Tougher Controls on Pathogen Research

In the 8 years since the 2001 anthrax letter attacks, new biocontainment labs have sprung up around the United States as part of a massive national effort to counter bioterrorism threats. Now, a crescendo of concerns over the security of these labs, voiced by lawmakers and experts at two congressional hearings last week, threatens to slow the booming biodefense industry.

Both hearings—in the House of Representatives and in the Senate—featured testimony from Nancy Kingsbury of the Government Accountability Office (GAO), whose agency released a report on 21 September that calls for entrusting a single federal entity with determining whether any more biocontainment labs are needed; what standards of design, maintenance, and operation such labs must meet; and how they should be overseen. Those recommendations dovetail with a bill introduced by senators Joe Lieberman (I-CT) and Susan Collins (R-ME) on 9 September, under which the Department of

Homeland Security (DHS) would be charged with establishing and enforcing new security standards for biocontainment labs. Currently, labs where researchers work with select agents—a subset of the broader universe of dangerous pathogens and toxins—are overseen by the select agent program, administered by the Department of Health and Human Services (HHS) and the U.S. Department of Agriculture (USDA).

GAO does not think the current system is adequate. "The number of BSL [biosafety level]-3 and BSL-4 labs has been proliferating at a considerable rate since the anthrax attacks because the government has been throwing money at this kind of research, but there's nobody in charge," Kingsbury told *Science*. She says the number of labs registered under the select agent program has more than tripled since 2004, to 1362 as of 2008.

"We need to know whether we need more of these labs or whether we already have too many," Kingsbury said. She was especially

concerned that an increase in the number of researchers working with hazardous pathogens would "inevitably" lead to an amplified risk of a bioterrorist attack perpetrated by a scientist working at a biocontainment facility. (In August 2008, the Federal Bureau of Investigation implicated U.S. Army researcher Bruce Ivins in the 2001 anthrax attacks [*Science*, 8 August 2008, p. 754].)

At the Senate hearing, lawmakers and officials from the Department of Defense and DHS spoke favorably of a tiered system for pathogens in which facilities and researchers dealing with the most dangerous microorganisms would be subject to the most stringent controls. The bill introduced by Lieberman and Collins calls for exactly such a classification. Under it, over a dozen pathogens, including anthrax, smallpox, and ebola, would be governed by tougher security standards than are required by the select agent rules. Rules for a second tier of select agents would be more lenient than they are now. Labs working

CREDIT: ADAPTED FROM J. KNIGHT ET AL., BULL. AMER. METEOR. SOC., 90 (SUPPL.), S22-S23 (AUGUST 2009)

way in the 700 years of simulation, about 17 separate 10-year intervals had temperature trends resembling that of the past decade—that is, more or less flat.

From this result, the group concludes that the model can reproduce natural jostlings of the climate system—perhaps a shift in heat-carrying ocean currents—that can cool the world and hold off greenhouse warming for a decade. But natural climate variability in the model has its limits. Pauses as long as 15 years are rare in the simulations, and “we expect that [real-world] warming will resume in the next few years,” the Hadley Centre group writes. And that resumption could come as a bit of a jolt, says Adam Scaife of the group, as the temperature catches up with the greenhouse gases added during the pause.

Pinning the pause on natural variability makes sense to most researchers. “That goes without saying,” writes climate researcher Stefan Rahmstorf of Potsdam Institute for Climate Impact Research in Germany by e-mail. “We’ve made [that point] several times on RealClimate,” a blog. Solar physicist Judith Lean of the Naval Research Laboratory in Washington, D.C., and climate modeler David Rind of GISS reached the same conclusion in a peer-reviewed 15 August paper in *Geophysical Research Letters*. They broke down recent

temperature variation into components attributable to greenhouse gases, pollutant aerosols, volcanic aerosols, El Niño/La Niña, and solar variability. Combined, those influences explain all of the observed variability, by Lean and Rind’s accounting. But unlike the Hadley Centre’s model-based analysis, this assessment attributes a good deal of climate variability to variability in solar activity. That’s because most models can’t translate solar variability into climate variability the way the actual climate system can (*Science*, 28 August, p. 1058), Rind says.

Researchers may differ about exactly what’s behind recent natural climate variability, but they agree that no sort of natural variability can hold off greenhouse warming much longer. “Our prediction is that if past is prologue, the solar component will turn around and lead to rapid warming in the next 5 years,” says Rind. Climate modeler David Smith of the Hadley Centre, who was not involved in the *State of the Climate* analysis, says his group’s climate model forecasts—made much the way weather forecasts are made—are still calling for warming to resume in the next few years as ocean influences reverse (*Science*, 10 August 2007, p. 746). Whether that’s in time to boost climate negotiations is anyone’s guess.

—RICHARD A. KERR

with a third tier of pathogens would have to register with the government to help coordinate a rapid response in case of an outbreak.

Many in the biodefense research community oppose more regulations or a change from the status quo. Ronald Atlas of the University of Louisville in Kentucky, who testified before the House panel on behalf of the American Society for Microbiology (ASM), says he supports the idea of tiering but wants HHS and USDA to remain in charge. “There is concern that the expertise at DHS is not sufficient in the life sciences,” Atlas says. Gerald Epstein of the Center for Strategic and International Studies in Washington, D.C., says that DHS’s system of ensuring the reliability of its own employees is so “dysfunctional and byzantine” that “it does not have any business” screening researchers working at other biocontainment labs.

As to how many labs are enough, Atlas says: “There would be value in having federal



Over capacity. GAO’s Kingsbury asks whether the United States already has too many biocontainment labs.

coordination with regard to the federal investment in such labs. But that sort of oversight should not interfere with the ability of private and academic institutions to construct containment labs using other sources of funding as long as those labs meet biosafety standards.”

ASM is urging Congress to hold off on passing any biosecurity legislation until an interagency task force on securing labs and countering the insider threat releases its recommendations later in the fall. Meanwhile, the biodefense enterprise is continuing to grow. In August, the U.S. Army Medical Research Institute of Infectious Diseases broke ground in Frederick, Maryland, for a new facility that will expand the institute’s BSL-3 and BSL-4 lab space. At the same time, a National Research Council panel is studying whether safety and security practices at USAMRIID—from where the anthrax attacks were purportedly launched—are adequate.

—YUDHIJIT BHATTACHARJEE

ScienceInsider



From the Science Policy Blog

The U.S. **Food and Drug Administration** has admitted that “unprecedented” pressure from New Jersey politicians led it to approve a **medical device** that its scientists said was flawed.

Roger Beachy has been chosen to head the **National Institute of Food and Agriculture**, which replaces the Cooperative State Research, Education, and Extension Service at the U.S. Department of Agriculture. Beachy is director of the Donald Danforth Plant Science Center in St. Louis, Missouri. **Arun Majumdar** has been nominated to run the new **Advanced Research Projects Agency—Energy** at the Department of Energy (DOE). The materials scientist comes from Lawrence Berkeley National Laboratory, and his new boss at DOE is his old boss at the lab—Energy Secretary Steven Chu.

A new online journal, *Nature Communications*, will allow authors to pay a fee so that their paper will be freely available online the moment it’s published. The journal will cover all disciplines and debuts this month.

A lavishly equipped graduate university with a multibillion-dollar endowment from the king officially opened last week along the shores of the Red Sea in Saudi Arabia. **King Abdullah University of Science and Technology** touted its Western-style approach to graduate education and research in a gala inauguration.

A case of **fabricated data** has caused upheavals at Switzerland’s top university, **ETH Zürich**. An internal investigation exonerated its vice president for research, in whose lab the fabrication occurred, but he nevertheless resigned his post. The investigation pointed to a former graduate student who is now suing ETH Zürich to block publication of the report.

Last week’s 1-day U.N. General Assembly **summit on climate change** gave **Chinese President Hu Jintao** a chance to tell the world that China stands ready to negotiate.

For more science policy news, visit blogs.sciencemag.org/scienceinsider.



The jungle. Many refugees in this French camp—now disbanded—sought asylum in Europe, particularly in the United Kingdom.

FORENSIC SCIENCE

Scientists Decry Isotope, DNA Testing of 'Nationality'

CAMBRIDGE, UNITED KINGDOM—Scientists are greeting with dismay a project to use DNA and isotope analysis of tissue from asylum-seekers to evaluate their nationality and help decide who can enter the United Kingdom. “Horrible,” “naïve,” and “flawed” are among the adjectives geneticists and isotope specialists *Science* contacted used to describe the “Human Provenance pilot project,” launched quietly in mid-September by the U.K. Border Agency. Their consensus: The project is not scientifically valid—or even sensible.

“My first reaction is this is wildly premature, even ignoring the moral and ethical aspects,” says Alec Jeffreys of the University of Leicester, who pioneered human DNA fingerprinting.

U.K. immigration policies have been under scrutiny recently as the number of people claiming asylum has soared and as French police in Calais last week cleared a camp of migrants hoping to make it across the English Channel. The existence of a DNA-based program to identify nationality was revealed in late September by the *Daily Mail* and *The Observer*, sparking protests from refugee advocates. *Science* has obtained Border Agency documents showing that isotope analyses of hair and nail samples will also be conducted “to help identify a person’s true country of origin.” The project

“is regrettable,” says Caroline Slocock, chief executive of Refugee and Migrant Justice headquartered in London. Although asylum-seekers are asked to provide tissue samples voluntarily, turning down a government request for tissue could be misinterpreted, she says, “so we believe [the program] should not be introduced at all.”

The Border Agency’s DNA-testing plans would use mouth swabs for mitochondrial DNA and Y chromosome testing, as well as analyses of subtle genetic variations called single-nucleotide polymorphisms (SNPs). One goal of the project is to determine whether asylum-seekers claiming to be from Somalia and fleeing persecution are actually from another African country such as Kenya. If successful, the Border Agency suggests its pilot project could be extended to confirming other nationalities. Yet scientists say the Border Agency’s goals confuse ancestry or ethnicity with nationality. David Balding, a population geneticist at Imperial College London, notes that “genes don’t respect national borders, as many legitimate citizens are migrants or direct descendants of migrants, and many national borders split ethnic groups.”

After reviewing the Border Agency’s plans, Jeffreys echoed those criticisms in an e-mail to *Science*: “The Borders Agency is clearly making huge and unwarranted assumptions about population structure in

Africa; the extensive research needed to determine population structure and the ability or otherwise of DNA to pinpoint ethnic origin in this region simply has not been done. Even if it did work (which I doubt), assigning a person to a population does not establish nationality—people move! The whole proposal is naïve and scientifically flawed.”

Another geneticist says the Forensic Science Service, a former government agency that has been privatized, requested his opinion earlier this year on how to develop a genetic assay to distinguish among East African populations. “I thought it was for forensic purposes, not border control,” says Christopher Phillips of the University of Santiago de Compostela in Spain, who with colleagues recently used a DNA sample to correctly infer the ancestry of a suspect in the 2004 train bombings in Madrid.

Mark Thomas, a geneticist of University College London who considers the Human Provenance program “horrible,” contends that even determining a person’s ancestry—as distinct from nationality—is more problematic than many believe. “mtDNA will never have the resolution to specify a country of origin. Many DNA ancestry testing companies have sprung up over the last 10 years, often based on mtDNA, but what they are selling is little better than genetic astrology,” he says. “Dense genomic SNP data does have

CREDIT: PHILIPPE HUGUEN/AFP/GETTY IMAGES

some resolution ... but not at a very local scale, and with considerable errors.”

Details of the plan to use isotope analyses in addition to DNA analyses have intensified skepticism. The plan is to look for ratios of certain isotopes in tissue that could be matched to ratios in the environment where a person was born or grew up. But isotope specialists point to a seemingly obvious flaw: There’s no scientifically accepted evidence that isotope signatures at birth or during childhood are still present in adult samples of constantly growing tissues such as hair and nails. At best, researchers say, those tissues reflect the past year or so of a person’s life. “It worries me as a scientist that actual peoples’ lives are being influenced based on these methods,” says Jane Evans, head of Science-based Archaeology at the National Environment Research Council Isotope Geosciences Laboratory in Nottingham.

Although the agency hasn’t detailed the isotopes it is examining, the use of hair and nail samples suggests that the tests will look at “lighter” element isotopes, such as those of hydrogen, oxygen, carbon, and nitrogen, all of which are incorporated into the keratin and other proteins as those tissues grow. Isotopes of strontium and other “heavier” elements incorporate into bones and teeth throughout life and some evidence suggests that strontium measurements can match people to geographic locales in which they were born, or at least grew up. In contrast, the lighter isotopes in tissues such as hair and nails being collected by the Border Agency are typically used to reveal recent diets and climatic conditions, not ethnicity. “I don’t think I could tell the difference between a Kenyan and a Somali,” says Tamsin O’Connell of the University of Cambridge in the United Kingdom, an archaeologist who specializes in studying light isotopes from soft tissues.

O’Connell, Evans, and others say they’re puzzled that one Border Agency document titled “Nationality-Swapping” uses the notorious “Adam Torso” case as a proof of principle for employing isotope analysis. In this highly publicized murder in 2001, only the mutilated torso of a teenager was found in the Thames river. Using isotope analysis, “the child’s body was traced to a small Nigerian town in an area about 100 x 50 km wide,” a Border Agency document states. (To read the documents describing the program and hear more reaction, follow the story at *ScienceInsider*.) The document notes, however, that the analysis was

done on bones, not on hair and teeth. “It’s like adding 2 and 2 and getting 3½,” says Jessica Pearson of the University of Liverpool, who uses isotope signatures from fossils to examine the diet of ancient humans. Pearson also points out that the forensic methods used in the Adam Torso case are impossible to evaluate because they still haven’t been described in a scientific publication or discussed in court.

Having their fate rest on unproven methods is particularly dangerous for asylum-seekers in the United Kingdom, notes Phillips, because, unlike criminal defendants, they have limited or no rights to challenge evidence or appeal. “You can’t parachute in a technique if it isn’t properly validated,” he says.

The Border Agency says only asylum-seekers who have already failed linguistic tests—another contested method of determining nationality—will be asked to provide mouth swabs, hair and nail samples. It also released a written response to scientific criticisms, which said: “Ancestral DNA testing will not be used

alone but will combine with language analysis, investigative interviewing techniques and other recognized forensic disciplines. The results of the combination of these procedures may indicate a person’s possible origin and enable the UKBA to make further enquiries leading to the return of those intending on abusing the

U.K.’s asylum system. This project is working with a number of leading scientists in this field who have studied differences in the genetic backgrounds of various population groups.”

The Border Agency has not yet responded to a request to identify the scientists it is working with, nor has it cited any scientific papers that validate its DNA and isotope methods. It’s also not clear who is conducting the DNA and isotope analyses for the Border Agency. Evans says her lab, which is arguably the United Kingdom’s leading academic center for isotope studies, is not involved. Several researchers say they suspect private labs are doing most of the work—and they question if such labs have been properly vetted for reliability. Among their many concerns, some scientists also worry that statistical uncertainties may be overlooked.

A Border Agency spokesperson defended its Human Provenance program as a “small pilot at the moment. It’s in its baby stages. We want to get feedback.” They’re getting plenty of that from outraged scientists. “I’d hate to see asylum decisions made [with these methods]. We’re dealing with people’s lives,” says Pearson.

—JOHN TRAVIS

“My first reaction is this is wildly premature, even ignoring the moral and ethical aspects.”

—ALEC JEFFREYS,
UNIVERSITY OF LEICESTER

ScienceNOW.org

From *Science’s* Online Daily News Site

The Upside of Recessions

You’ve lost your job, your house, and your savings. But, hey, you still have your health, right? Actually, you probably do—and it may even be improving. Researchers have found that, historically, Americans were healthier during the Great Depression and other economic downturns than they were during periods of prosperity. And they say the trend may still hold true today. <http://bit.ly/3kBH8L>

Dylan to Darwin: Don’t Look Back

Evolution doesn’t make U-turns, according to a new study of proteins. The research shows that simply reversing selective pressure won’t make a biomolecule revert to an earlier form. The finding confirms a much-debated biological law that, evolutionarily speaking, there’s no going back. <http://bit.ly/2sXUxo>

Wolf in Coyote’s Clothing

Farmers depend on hybrid vigor for improved crop yields, as seeds produced from different strains of, say, corn, can lead to superior crops. Hybrid vigor seems to have worked for coyotes in the Northeastern United States as well, according to a genetic study and physical analysis of the animals: Coyotes in this part of the United States are bigger than their western counterparts because, as their ancestors migrated into the territory, they mated with wolves along the way. <http://bit.ly/vvsqU>



Taking the Tally of Curious Triangles

What do the numbers 5, 6, 7, 13, 14, 15, 20, 21, 22, and 23 have in common? They’re all “congruent” numbers, that is, numbers related to the areas of certain triangles. Even if that answer didn’t leap to mind, you may be intrigued to know that mathematicians have now cataloged the congruent numbers—which are easy to define but not so easy to spot—up to a trillion. <http://bit.ly/3uj8bt>

Read the full postings, comments, and more on scienown.sciencemag.org.

On the Origin of Ecological Structure



ON 23 JUNE 1802, PRUSSIAN NATURALIST

Alexander von Humboldt attempted to reach the summit of Mount Chimborazo, the highest peak in the northern Andes. Bleeding, his beard caked with ice, the 33-year-old Humboldt worked his way along a 12-centimeter-wide ridge only to be blocked by a cliff some 400 meters from the top. Humboldt's barometer indicated 5878 meters—a climbing record unbeaten for decades and one that brought him international fame.

The lasting impact of the trip, however, came from his explorations of somewhat less lofty terrain. Having studied Mount Chimborazo and nearby peaks for months, Humboldt assembled the first comprehensive treatise—*Essay on the Geography of Plants*—on how vegetation varies with altitude, climate, soil, and other factors. The work was a groundbreaking exploration of the physical underpinnings of ecological structure: what determines the species that make up a community and their relative abundance.

More than a half-century later, Charles Darwin quietly conducted experiments in his garden at Down House that were even more seminal. Examining a patch of unkempt lawn as it went to seed, Darwin observed that the species changed through time: “more vigorous plants gradually kill the less vigorous, though fully grown plants,” he wrote; nine of the original 20 species eventually disappeared. It was a compelling demonstration of competition, which became a cornerstone,

albeit a controversial one, for community structure, and Darwin included the experiment in *On the Origin of Species*. “What a wondrous problem it is,” Darwin wrote to the botanist Joseph Hooker in 1857, “what a play of forces, determining the kind and proportion of each plant in a square yard of turf!”

Ever since, ecologists have wrestled with understanding what dictates the kinds and proportions of organisms in communities ranging from meadows to montane forests. How these forces set up communities has “arguably been one of the most primary questions driving ecological science since its origins,” says Brian Enquist of the University of Arizona, Tucson. Competition, predation, disturbance, and other factors have a heavy hand, and new research is showing the influential role of evolution as well. “You can’t understand the assembly process if you don’t think about evolution,” says Jeannine Cavender-Bares of the University of Minnesota, Twin Cities.

Despite these achievements, there is still no consensus on the relative importance of the various forces. Darwin and many later ecologists emphasized competition among species, but proponents of a controversial theory of biodiversity that assumes competition has no impact argue that immigration and other random demographic events can account for much of the apparent makeup of communities. As a result, ecologists have a long way to go to come up with formulas that predict how communities might arise and change. Yet the ability to make predictions is important for the restoration and management of ecosystems impacted by invasive species or climate change.

Many forces

Species abundance and composition—i.e., structure—may be the salient feature of a biological community. A tropical rainforest, for example, is physically dominated by tall, broad-leaved trees with several layers of trees underneath adapted to lower light. Woody vines and epiphytes dangle from the branches, and shade-tolerant shrubs dot the forest floor. Even though the particular species vary from place

to place, wet tropical forests still exist as recognizable entities on four continents. A combination of physical and biological forces organizes species into these predictable communities.

Following Humboldt’s lead, scientists in the 19th century assembled evidence that the composition of communities depends on physical factors such as climate and soil chemistry. Today, ecologists call these factors “environmental filters” that broadly determine which species can live where. For example, forests in the eastern United States are rich in sugar maples in the north but gradually become dominated by oaks and hickories to the south as temperature rises. Hemlock and beech trees disappear to the west as conditions generally become drier.

On a global scale, the importance of physical factors varies with latitude, according to conventional thinking, popularized by Theodore Dobzhansky in 1950. Stress from cold and freezing limits diversity at high latitudes, according to this widely established view, whereas species diversity in the tropics is capped by another major driver, biological interactions.

But to what degree are local patterns driven by the direct influence of climate versus biological interactions such as competition? “Answering this question is critical for our ability to predict shifts in natural communities due to global climate change,” says Nicholas Gotelli of the University of Vermont, Burlington.

THE YEAR OF DARWIN

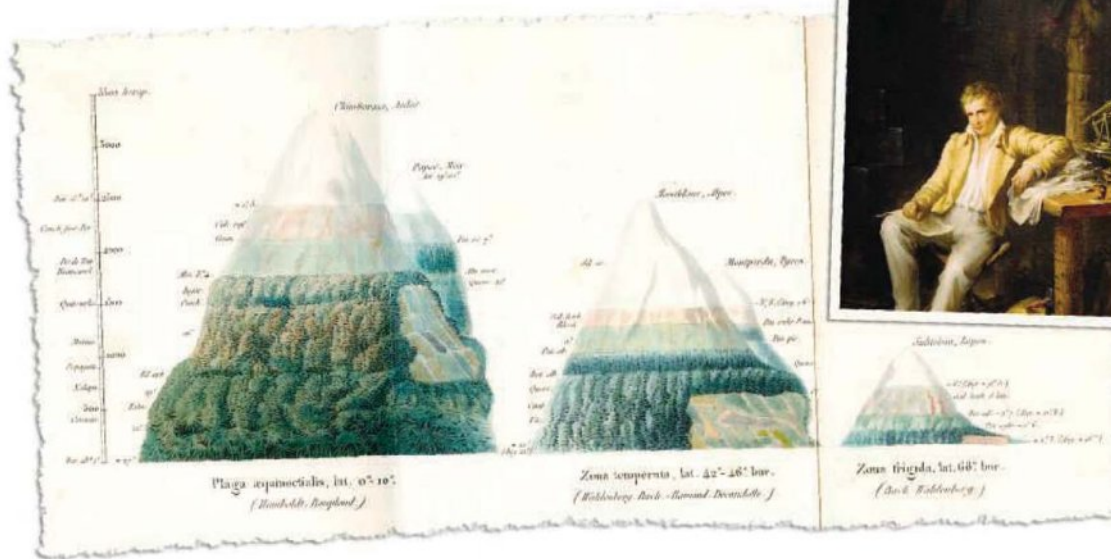


This essay is the 10th in a monthly series. For more on evolutionary topics online, see the Origins blog at blogs.sciencemag.org/origins. For more on ecological structure, listen to a podcast by author Erik Stokstad at www.sciencemag.org/multimedia/podcast.

It’s long been clear that biological interactions—competition, predation, and so on—can be big players. In the 1930s, Soviet microbiologist Georgii Gause conducted influential research into how competition sets up communities. Gause studied mixtures of three species of the protist *Paramecium* that were provided with one or two kinds of food: yeast, bacteria, or both. The experiments revealed that one species of *Paramecium* would always drive the others extinct if they had to compete for the same resource. This led to the principle of competitive exclusion and eventually to the idea that species that are ecologically too similar

cannot coexist. Although ecologists now know that the natural world is more complicated—there are ways for similar species to coexist—the principle had a major impact on thinking about the structuring of communities.

Over subsequent decades, ecologists recognized that predators, too, can strongly shape



In the zone. Alexander von Humboldt (above, left) compared the influence of elevation on plant communities on Mount Chimborazo (left), Mont Blanc, and Sulitelma.

communities. Working in rocky tide pools of the Pacific Northwest, for example, Robert Paine of the University of Washington, Seattle, proposed in 1966 that species diversity is controlled by keystone predators. By eating species that are strong competitors within the food web, keystone predators help weaker competitors persist. Sea stars, for example, feed on mussels and keep them from crowding out barnacles and algae.

A species doesn't have to be a predator or a competitor to have a profound effect. By altering the physical environment, some species influence which organisms live where. When beavers build dams they flood land, providing new aquatic habitat for fish and amphibians. Corals create a three-dimensional space full of places to hide, eat, and live for a wide variety of marine life. Thus one organism can facilitate the settlement or success of another.

Debating competition

Although certain ecosystems present clear examples of how biological interactions shape communities, coming up with general principles has been much more difficult. Arguments have raged for decades about the relative importance of factors such as competition, predation, and chance events: colonization, for example. A major debate about competition and how to spot it kicked off in 1975, when Jared Diamond of the University of California, Los Angeles, proposed seven broad patterns of species distributions, which he dubbed "assembly rules," for communities. The first rule was that only some of all the possible combinations of species actually coexist in nature. Diamond identified several instances of "forbidden species combinations," based on literature and fieldwork on fruit-eating birds living in the Bismarck Archipelago and Solomon Islands near New

Guinea. For example, the black honeyeater (*Myzomela pammelaena*) lives on 23 of the 41 surveyed islands in the Bismarck Archipelago, but not on any of the 14 islands inhabited by the black sunbird (*Nectarinia sericea*). Both birds are about the same size and use curved bills to sip nectar, and Diamond noted that competition affects their distribution.

But without any experimental evidence or strong statistical tests, it was a bold leap to conclude that competition was a major force structuring island communities. Another interpretation came from Daniel Simberloff and Edward Connor, then at Florida State University, Tallahassee. Starting in 1979, they argued that patterns of species distribution on these islands appeared to be random. "The pattern was eyeballed," Simberloff says of Diamond's results.

The disagreement continues to this day. Working with James Sanderson of the Wildlife Conservation Network in Los Altos, California, and Stuart Pimm of Duke University in Durham, North Carolina, Diamond published a new analysis of the bird species of the Bismarck and Solomon Islands in *Evolutionary Ecology Research* in July. By using what they say are more sophisticated statistical tests, the team verified that the patterns of species combinations identified by Diamond in 1975 were indeed highly unlikely to be due to chance. Although chance may determine which species end up colonizing an island, interspecific competition then tends to keep out ecologically similar species, Pimm says. Simberloff, meanwhile, has a paper in press in which he finds that the

patterns in the same data are better explained by the historical and chance factors that control how birds disperse than by competition.

A handful of so-called assembly rules have been proposed since Diamond's early work popularized the search for these patterns. But local communities are so varied that it seems difficult to extrapolate from one to another. "I think what we're going to find out is that assembly rules are vague, gentle constraints," says Evan Weiher of the University of Wisconsin, Eau Claire.

In 1997, Stephen Hubbell, now at the University of Georgia, Athens, proposed an alternative to assembly driven by competition or other biological interactions. Instead, Hubbell suggested, the abundance and diversity of species in a community is determined mainly by random dispersal, speciation, and extinction. The idea, which he dubbed the "unified neutral theory of biodiversity," makes a radical assumption: It considers all organisms of the same trophic level (plants, say, or herbivores) as demographically identical; that is, each organism in a particular level has about the same chance of reproducing, dying, migrating, or giving rise to a new species. Testing the idea on a 50-hectare plot of tropical forest in

"I think what we're going to find out is that assembly rules are vague, gentle constraints."

—Evan Weiher, University of Wisconsin, Eau Claire

Panama, Hubbell showed that the model predicted the species richness and relative abundance in the area. Hubbell doesn't dispute that some species differ in their ability to compete, but competition wasn't really an important factor in determining what plants grew where, he noted.

Neutral theory has generated a lot of interest, especially among theorists, and controversy. On the one hand, researchers find it appealing because of its simplicity and the fact that it provides predictions for many kinds of communities. But many ecologists remain skeptical of the assumption that species are essentially equivalent in how they function in the community. A recent paper by Nathan Kraft of the University of California, Berkeley, and others even challenges the adequacy of neutral theory in tropical forests, where it was first proposed, and instead makes a case for functional differences among species, and perhaps competition, as contributing factors (*Science*, 24 October 2008, p. 580).

Researchers also point out that biological interactions and “neutral” factors, such as the stochastic effects of dispersal, aren’t mutually exclusive. Both can sometimes happen in different ways simultaneously. For example, in a 2005 paper in *Ecology Letters*, Tadashi Fukami, now of Stanford University in Palo Alto, California, and Wim Van der Putten of Wageningen University in the Netherlands described a 9-year experiment with plants on abandoned farmland. They found that small communities of plants ended up with the same array of functional traits, such as whether a seed is dispersed by an animal or whether the plant is a perennial, indicating that biological interactions were determining what kinds of plant species could successfully establish. But the particular species that showed up were essentially random selections from the regional species pool, a result consistent with neutral theory.

Predicting the future

Ecologists would also like to know how the structure of communities will shift through time. Moreover, with all the changes humans have made to the environment, restoration ecologists and conservation biologists want to predict the future of these altered communities.

Communities are typically in flux, with some species disappearing and new ones taking hold after relatively minor disturbances.

In forests, for example, when a storm knocks down a stand of trees, more light reaches the forest floor. Small, short-lived flowering plants move in, then shrubs, and tree seedlings that within a decade or so begin to shade out the herbaceous plants.

A pioneer in the study of this process, called succession, was Frederic Clements of the University of Nebraska, Lincoln. He thought succession would inevitably lead to a particular climax community, and the system would remain in equilibrium until a disturbance started the cycle over again. Although this seems to be largely true for some plant communities, such as temperate

starfish that keep the mollusks in check.

Although it’s not known how common alternate stable states might be, the concept has important implications for restoration ecologists, who want to know whether degraded habitat will repair itself or whether it needs intervention to prevent it from falling into an undesirable new state. But there are so

many variables that predicting what will happen is difficult.

That same limitation applies to assembly rules. In a study of salt marshes published in March in *Ecological Applications*, a group of ecologists found that although physical stress chiefly determines the distribution of plants in a California marsh, competition is the main force in similar salt-marsh communities in Chile. The finding sug-



Diversity. Structure results from many sources, including predators such as star fish and habitat-building organisms such as coral; physical factors such as temperature also influence where species, like these trees in Quebec, Canada, can thrive.



forests, other types of communities appear to behave differently.

In rocky intertidal communities in the Gulf of Maine, for example, the community can shift between two alternate states, dominated by either algae or mussels. Peter Petraitis of the University of Pennsylvania and Steve Dudgeon of California State University, Northridge, scraped all the life off coastal rocks in the Gulf of Maine to create patches of open habitat. As they and colleagues reported in *Oecologia* in April, the identity of the new community depended on which organism got there first. If mussel larvae landed, they grew faster than the algae. But if the algae had enough time to get started, they sheltered mussel predators like

gests that general rules won’t provide conservation biologists with easy shortcuts when they’re trying to save or restore unstudied communities.

But Brian Silliman of the University of Florida, Gainesville, a study author, says that ecology still provides valuable insights, such as the potential impact of removing keystone predators. “We can generalize in large ways,” he says. Pimm agrees that some broad principles do exist. Although general community rules may not always provide fine-scale predictions about how a community will assemble, he says, they are “hugely useful and critical for conservation.”

—ERIK STOKSTAD

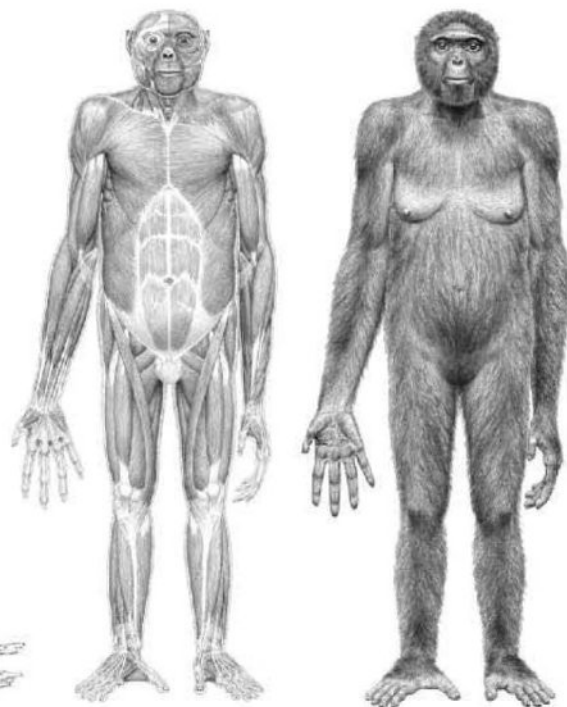
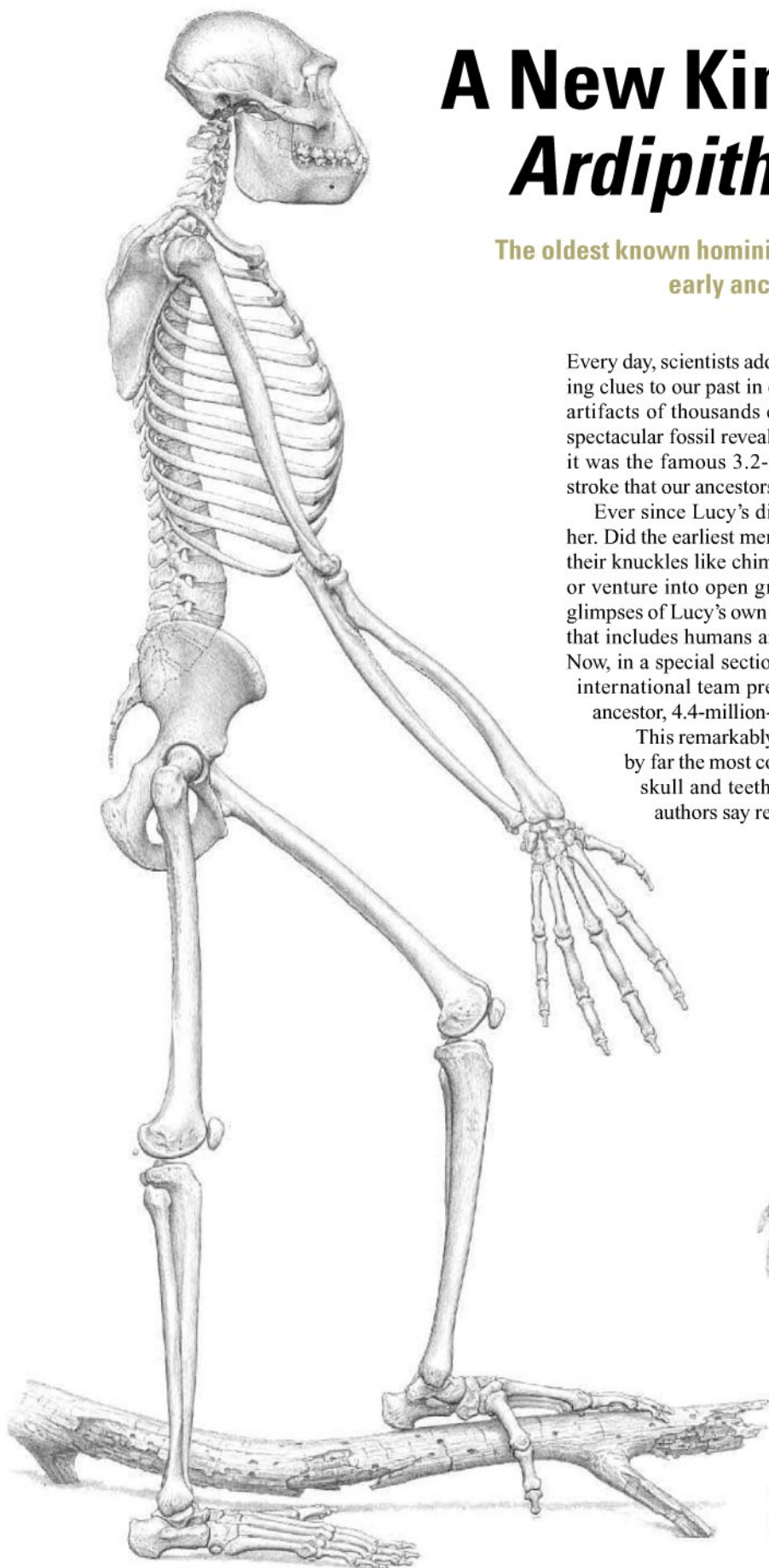
A New Kind of Ancestor: *Ardipithecus* Unveiled

The oldest known hominin skeleton reveals the body plan of our very early ancestors and the upright origins of humankind

Every day, scientists add new pages to the story of human evolution by deciphering clues to our past in everything from the DNA in our genes to the bones and artifacts of thousands of our ancestors. But perhaps once each generation, a spectacular fossil reveals a whole chapter of our prehistory all at once. In 1974, it was the famous 3.2-million-year-old skeleton “Lucy,” who proved in one stroke that our ancestors walked upright before they evolved big brains.

Ever since Lucy’s discovery, researchers have wondered what came before her. Did the earliest members of the human family walk upright like Lucy or on their knuckles like chimpanzees and gorillas? Did they swing through the trees or venture into open grasslands? Researchers have had only partial, fleeting glimpses of Lucy’s own ancestors—the earliest hominins, members of the group that includes humans and our ancestors (and are sometimes called hominids). Now, in a special section beginning on page 60 and online, a multidisciplinary international team presents the oldest known skeleton of a potential human ancestor, 4.4-million-year-old *Ardipithecus ramidus* from Aramis, Ethiopia.

This remarkably rare skeleton is not the oldest putative hominin, but it is by far the most complete of the earliest specimens. It includes most of the skull and teeth, as well as the pelvis, hands, and feet—parts that the authors say reveal an “intermediate” form of upright walking, consid-



From the inside out. Artist's reconstructions show how Ardi's skeleton, muscles, and body looked and how she would have moved on top of branches.

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ered a hallmark of hominins. “We thought Lucy was the find of the century but, in retrospect, it isn’t,” says paleoanthropologist Andrew Hill of Yale University. “It’s worth the wait.”

To some researchers’ surprise, the female skeleton doesn’t look much like a chimpanzee, gorilla, or any of our closest living primate relatives. Even though this species probably lived soon after the dawn of humankind, it was not transitional between African apes and humans. “We have seen the ancestor, and it is not a chimpanzee,” says paleoanthropologist Tim White of the University of California, Berkeley, co-director of the Middle Awash research group, which discovered and analyzed the fossils.

Instead, the skeleton and pieces of at least 35 additional individuals of *Ar. ramidus* reveal a new type of early hominin that was neither chimpanzee nor human. Although the team suspects that *Ar. ramidus* may have given rise to Lucy’s genus, *Australopithecus*, the fossils “show for the first time that there is some new evolutionary grade of hominid that is not *Australopithecus*, that is not *Homo*,” says paleontologist Michel Brunet of the College de France in Paris.

In 11 papers published in this issue and online, the team of 47 researchers describes how *Ar. ramidus* looked and moved. The skeleton, nicknamed “Ardi,” is from a female who lived in a woodland (see sidebar, p. 40), stood about 120 centimeters tall, and weighed about 50 kilograms. She was thus as big as a chimpanzee and had a brain size to match. But she did not knuckle-walk or swing through the trees like living apes. Instead, she walked upright, planting her feet flat on the ground, perhaps eating nuts, insects, and small mammals in the woods.

She was a “facultative” biped, say the authors, still living in both worlds—upright on the ground but also able to move on all fours on top of branches in the trees, with an opposable big toe to grasp limbs. “These things were very odd creatures,” says paleoanthropologist Alan Walker of Pennsylvania State University, University Park. “You know what Tim [White] once said: If you wanted to find something that moved like these things, you’d have to go to the bar in *Star Wars*.”

Most researchers, who have waited 15 years for the publication of this find, agree that Ardi is indeed an early hominin. They praise the detailed reconstructions needed to piece together the crushed bones. “This is an extraordinarily impressive work of reconstruction and description, well worth waiting for,” says paleoanthropologist David Pilbeam of Harvard University. “They did this job very, very well,” agrees neurobiologist Christoph Zollikofer of the University of Zurich in Switzerland.

But not everyone agrees with the team’s interpretations about how *Ar. ramidus* walked upright and what it reveals about our ancestors.

“The authors ... are framing the debate that will inevitably follow,” because the description and interpretation of the finds are entwined, says Pilbeam. “My first reaction is to be skeptical about some of the conclusions,” including that human ancestors never went through a chimpanzee-like phase. Other researchers are focusing intently on the lower skeleton, where some of the anatomy is so primitive that they are beginning to argue over just what it means to be “bipedal.” The pelvis, for example, offers only “circumstantial” evidence for upright walking, says Walker. But however the debate about Ardi’s locomotion and identity evolves, she provides the first hard evidence that will inform and constrain future ideas about the ancient hominin bauplan.

Online

sciencemag.org

Podcast interview
with author
Ann Gibbons on
Ardipithecus and
fieldwork in the Afar.

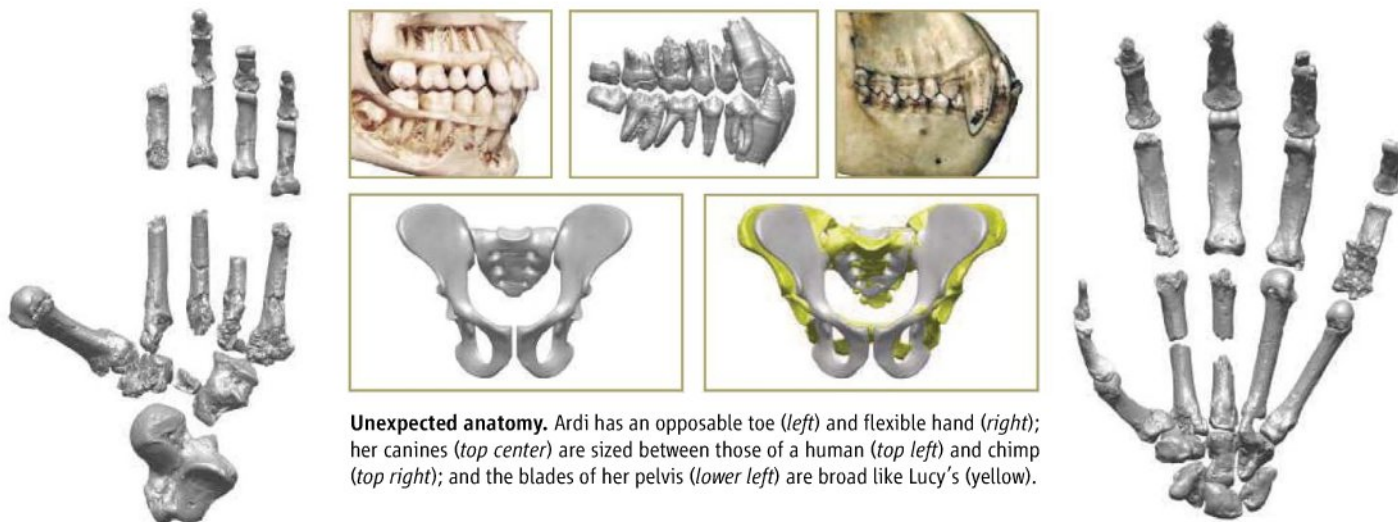
Digging it

The first glimpse of this strange creature came on 17 December 1992 when a former graduate student of White’s, Gen Suwa, saw a glint among the pebbles of the desert pavement near the village of Aramis. It was the polished surface of a tooth root, and he immediately

knew it was a hominin molar. Over the next few days, the team scoured the area on hands and knees, as they do whenever an important piece of hominin is found (see story, p. 41), and collected the lower jaw of a child with the milk molar still attached. The molar was so primitive that the team knew they had found a hominin both older and more primitive than Lucy. Yet the jaw also had derived traits—novel evolutionary characters—shared with Lucy’s species, *Au. afarensis*, such as an upper canine shaped like a diamond in side view.

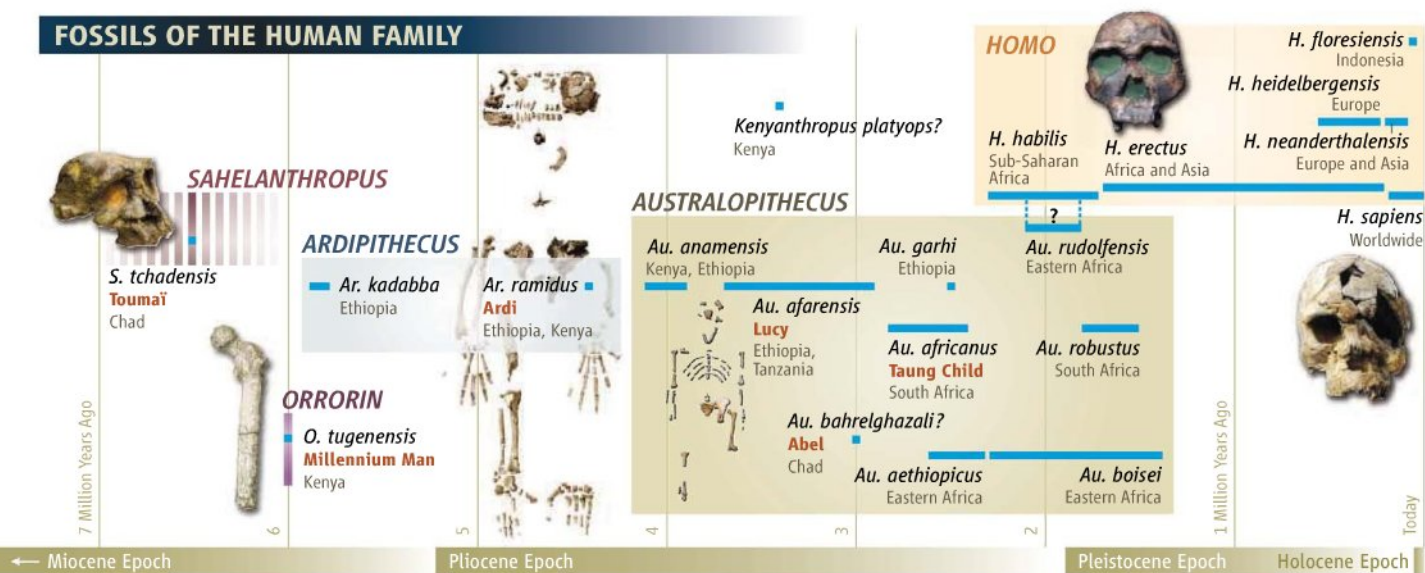
The team reported 15 years ago in *Nature* that the fragmentary fossils belonged to the “long-sought potential root species for the Hominidae.” (They first called it *Au. ramidus*, then, after finding parts of the skeleton, changed it to *Ar. ramidus*—for the Afar words for “root” and “ground.”) In response to comments that he needed leg bones to prove *Ar. ramidus* was an upright hominin, White joked that he would be delighted with more parts, specifically a thigh and an intact skull, as though placing an order.

Within 2 months, the team delivered. In November 1994, as the fossil hunters crawled up an embankment, Berkeley graduate student Yohannes Haile-Selassie of Ethiopia, now a paleoanthropologist at the Cleveland Museum of Natural History in Ohio, spotted two pieces of a bone from the palm of a hand. That was soon followed by pieces of a pelvis; leg, ankle, and foot bones; many of the bones of the hand and arm; a lower jaw with teeth—and a cranium. By January 1995, it was apparent that they had made the rarest of rare finds, a partial skeleton.



Unexpected anatomy. Ardi has an opposable toe (left) and flexible hand (right); her canines (top center) are sized between those of a human (top left) and chimp (top right); and the blades of her pelvis (lower left) are broad like Lucy’s (yellow).

FOSSILS OF THE HUMAN FAMILY



Filling a gap. *Ardipithecus* provides a link between earlier and later hominins, as seen in this timeline showing important hominin fossils and taxa.

It is one of only a half-dozen such skeletons known from more than 1 million years ago, and the only published one older than Lucy.

It was the find of a lifetime. But the team's excitement was tempered by the skeleton's terrible condition. The bones literally crumbled when touched. White called it road kill. And parts of the skeleton had been trampled and scattered into more than 100 fragments; the skull was crushed to 4 centimeters in height. The researchers decided to remove entire blocks of sediment, covering the blocks in plaster and moving them to the National Museum of Ethiopia in Addis Ababa to finish excavating the fossils.

It took three field seasons to uncover and extract the skeleton, repeatedly crawling the site to gather 100% of the fossils present. At last count, the team had cataloged more than 110 specimens of *Ar. ramidus*, not to mention 150,000 specimens of fossil plants and animals. "This team seems to suck fossils out of the earth," says anatomist C. Owen Lovejoy of Kent State University in Ohio, who analyzed the post-cranial bones but didn't work in the field. In the lab, he gently unveils a cast of a tiny, pea-sized sesamoid bone for effect. "Their obsessiveness gives you—this!"

White himself spent years removing the silty clay from the fragile fossils at the National Museum in Addis Ababa, using brushes, syringes, and dental tools, usually under a microscope. Museum technician Alemu Ademassu made a precise cast of each piece, and the team assembled them into a skeleton.

Meanwhile in Tokyo and Ohio, Suwa and Lovejoy made virtual reconstructions of the crushed skull and pelvis. Certain fossils were taken briefly to Tokyo and scanned with a custom micro-computed tomography (CT) scanner that could reveal what was hidden inside the bones and teeth. Suwa spent 9 years mastering the technology to reassemble the fragments of the cranium into a virtual skull. "I used 65 pieces of the cranium," says Suwa, who estimates he spent 1000 hours

on the task. "You go piece by piece."

Once he had reassembled the pieces in a digital reconstruction, he and paleoanthropologist Berhane Asfaw of the Rift Valley Research Service in Addis Ababa compared the skull with those of ancient and living primates in museums worldwide. By March of this year, Suwa was satisfied with his 10th reconstruction. Meanwhile in Ohio, Lovejoy made physical models of the pelvic pieces based on the original fossil and the CT scans, working closely with Suwa. He is also satisfied that the 14th version of the pelvis is accurate. "There was an *Ardipithecus* that looked just like that," he says, holding up the final model in his lab.



Fossil finders. Tim White and local Afar fossil hunters pool their finds after scouring the hillside at Aramis.

As they examined Ardi's skull, Suwa and Asfaw noted a number of characteristics. Her lower face had a muzzle that juts out less than a chimpanzee's. The cranial base is short from front to back, indicating that her head balanced atop the spine as in later upright walkers, rather than to the front of the spine, as in quadrupedal apes. Her face is in a more vertical position than in

Putting their heads together

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chimpanzees. And her teeth, like those of all later hominins, lack the daggerlike sharpened upper canines seen in chimpanzees. The team realized that this combination of traits matches those of an even older skull, 6-million to 7-million-year-old *Sahelanthropus tchadensis*, found by Brunet's team in Chad. They conclude that both represent an early stage of human evolution, distinct from both *Australopithecus* and chimpanzees. "Similarities with *Sahelanthropus* are striking, in that it also represents a first-grade hominid," agrees Zollikofer, who did a three-dimensional reconstruction of that skull.

Another, earlier species of *Ardipithecus*—*Ar. kadabba*, dated from 5.5 million to 5.8 million years ago but known only from teeth and bits and pieces of skeletal bones—is part of that grade, too. And *Ar. kadabba*'s canines and other teeth seem to match those of a third very ancient specimen, 6-million-year-old *Orrorin tugenensis* from

Kenya, which also has a thighbone that appears to have been used for upright walking (*Science*, 21 March 2008, p. 1599). So, “this raises the intriguing possibility that we’re looking at the same genus” for specimens now put in three genera, says Pilbeam. But the discoverers of *O. tugenensis* aren’t so sure. “As for Ardi and *Orrorin* being the same genus, no, I don’t think this is possible, unless one really wants to accept an unusual amount of variability” within a taxon, says geologist Martin Pickford of the Collège de France, who found *Orrorin* with Brigitte Senut of the National Museum of Natural History in Paris.

Whatever the taxonomy of *Ardipithecus* and the other very ancient hominins, they represent “an enormous jump to *Australopithecus*,” the next hominin in line (see timeline, p. 38), says australopithecine expert William Kimbel of Arizona State University, Tempe. For example, although Lucy’s brain is only a little larger than that of *Ardipithecus*, Lucy’s species, *Au. afarensis*, was an adept biped. It walked upright like humans, venturing increasingly into more diverse habitats, including grassy savannas. And it had lost its opposable big toe, as seen in 3.7-million-year-old footprints at Laetoli, Tanzania, reflecting an irreversible commitment to life on the ground.

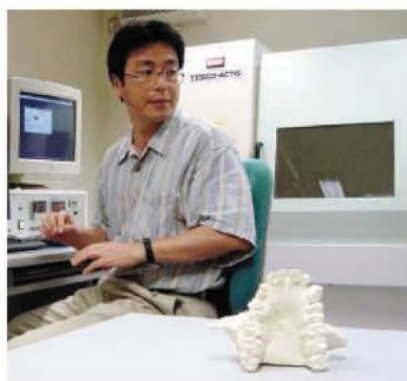
Lucy’s direct ancestor is widely considered to be *Au. anamensis*, a hominin whose skeleton is poorly known, although its shinbone suggests it walked upright 3.9 million to 4.2 million years ago in Kenya and Ethiopia. *Ardipithecus* is the current leading candidate for *Au. anamensis*’s ancestor, if only because it’s the only putative hominin in evidence between 5.8 million and 4.4 million years ago. Indeed, *Au. anamensis* fossils appear in the Middle Awash region just 200,000 years after Ardi.

Making strides

But the team is not connecting the dots between *Au. anamensis* and *Ar. ramidus* just yet, awaiting more fossils. For now they are focusing on the anatomy of Ardi and how she moved through the world. Her foot is primitive, with an opposable big toe like that used by living apes to grasp branches. But the bases of the four other toe bones were oriented so that they reinforced the forefoot into a more rigid lever as she pushed off. In contrast, the toes of a chimpanzee curve as flexibly as those in their hands, say Lovejoy and co-author Bruce Latimer of Case Western Reserve University in Cleveland. *Ar. ramidus* “developed a pretty good bipedal foot while at the same time keeping an opposable first toe,” says Lovejoy.

The upper blades of Ardi’s pelvis are shorter and broader than in apes. They would have lowered the trunk’s center of mass, so she could balance on one leg at a time while walking, says Lovejoy. He also infers from the pelvis that her spine was long and curved like a human’s rather than short and stiff like a chimpanzee’s. These changes suggest to him that *Ar. ramidus* “has been bipedal for a very long time.”

Yet the lower pelvis is still quite large and primitive, similar to African apes rather than hominins. Taken with the opposable big toe, and primitive traits in the hand and foot, this indicates that *Ar. ramidus* didn’t walk like Lucy and was still spending a lot of time in the trees. But it wasn’t suspending its body beneath branches like African apes or climbing vertically, says Lovejoy. Instead, it was a slow, careful



Dream team. Gen Suwa (left) in Tokyo focused on the skull; C. Owen Lovejoy (top right) in Kent, Ohio, studied postcranial bones; and Yohannes Haile-Selassie and Berhane Asfaw found and analyzed key fossils in Ethiopia.

climber that probably moved on flat hands and feet on top of branches in the midcanopy, a type of locomotion known as palmigrady. For example, four bones in the wrist of *Ar. ramidus* gave it a more flexible hand that could be bent backward at the wrist. This is in contrast to the hands of knuckle-walking chimpanzees and gorillas, which have stiff wrists that absorb forces on their knuckles.

However, several researchers aren’t so sure about these inferences. Some are skeptical that the crushed pelvis really shows the anatomical details needed to demonstrate bipedality. The pelvis is “suggestive” of bipedality but not conclusive, says paleoanthropologist Carol Ward of the University of Missouri, Columbia. Also, *Ar. ramidus* “does not appear to have had its knee placed over the ankle, which means that when walking bipedally, it would have had to shift its weight to the side,” she says. Paleoanthropologist William Jungers of Stony Brook University in New York state is also not sure that the skeleton was bipedal. “Believe me, it’s a unique form of bipedalism,” he says. “The postcranium alone would not unequivocally signal hominin status, in my opinion.” Paleoanthropologist Bernard Wood of George Washington University in Washington, D.C., agrees. Looking at the skeleton as a whole, he says, “I think the head is consistent with it being a hominin, ... but the rest of the body is much more questionable.”

All this underscores how difficult it may be to recognize and define bipedality in the earliest hominins as they began to shift from trees to ground. One thing does seem clear, though: The absence of many specialized traits found in African apes suggests that our ancestors never knuckle-walked.

That throws a monkey wrench into a hypothesis about the last common ancestor of living apes and humans. Ever since Darwin

Habitat for Humanity

ARAMIS, ETHIOPIA—A long cairn of black stones marks the spot where a skeleton of *Ardipithecus ramidus* was found, its bones broken and scattered on a barren hillside. Erected as a monument to an ancient ancestor in the style of an Afar tribesman's grave, the cairn is a solitary marker in an almost sterile zone, devoid of life except for a few spindly acacia trees and piles of sifted sediment.

That's because the Middle Awash research team sucked up everything in sight at this spot, hunting for every bit of fossil bone as well as clues to the landscape 4.4 million years ago, when *Ardipithecus* died here. "Literally, we crawled every square inch of this locality," recalls team co-leader Tim White of the University of California, Berkeley. "You crawl on your hands and knees, collecting every piece of bone, every piece of wood, every seed, every snail, every scrap. It was 100% collection." The heaps of sediment are all that's left behind from that fossil-mining operation, which yielded one of the most important fossils in human evolution (see main text, p. 36), as well as thousands of clues to its ecology and environment.

The team collected more than 150,000 specimens of fossilized plants and animals from nearby localities of the same age, from elephants to songbirds to millipedes, including fossilized wood, pollen, snails, and larvae. "We have crates of bone splinters," says White.

A team of interdisciplinary researchers then used these fossils and thousands of geological and isotopic samples to reconstruct *Ar. ramidus*'s Pliocene world, as described in companion papers in this issue (see p. 66 and 87). From these specimens, they conclude that Ardi lived in a woodland, climbing among hackberry, fig, and palm trees and coexisting with monkeys, kudu antelopes, and peafowl. Doves and parrots flew overhead. All these creatures prefer woodlands, not the open, grassy terrain often conjured for our ancestors.

The team suggests that *Ar. ramidus* was "more omnivorous" than chimpanzees, based on the size, shape, and enamel distribution of its teeth. It probably supplemented woodland plants such as fruits, nuts, and tubers with the occasional insects, small mammals, or bird eggs. Carbon-isotope studies of teeth from five individuals show that *Ar. ramidus* ate mostly woodland, rather than grassland, plants. Although *Ar. ramidus* probably ate

suggested in 1871 that our ancestors arose in Africa, researchers have debated whether our forebears passed through a great-ape stage in which they looked like proto-chimpanzees (*Science*, 21 November 1969, p. 953). This "troglodytan" model for early human behavior (named for the common chimpanzee, *Pan troglodytes*) suggests that the last common ancestor of the African apes and humans once had short backs, arms adapted for swinging, and a pelvis and limbs adapted for knuckle walking. Then our ancestors lost these traits, while chimpanzees and gorillas kept them. But this view has been uninformed by fossil evidence because there are almost no fossils of early chimpanzees and gorillas.

Some researchers have thought that the ancient African ape bauplan was more primitive, lately citing clues from fragmentary fossils of apes that lived from 8 million to 18 million years ago. "There's been growing evidence from the Miocene apes that the common ancestor may have been more primitive," says Ward. Now *Ar. ramidus* strongly supports that notion. The authors repeatedly

figs and other fruit when ripe, it didn't consume as much fruit as chimpanzees do today.

This new evidence overwhelmingly refutes the once-favored but now moribund hypothesis that upright-walking hominins arose in open grasslands. "There's so much good data here that people aren't going to be able to question whether early hominins were living in woodlands," says paleo-anthropologist Andrew Hill of Yale University. "Savannas had nothing to do with upright walking."

Geological studies indicate that most of the fossils were buried within a relatively short window of time, a few thousand to, at most, 100,000 years ago, says geologist and team co-leader Giday WoldeGabriel of the Los Alamos National Laboratory in New Mexico. During that sliver of time, Aramis was not a dense tropical rainforest with a thick canopy but a humid, cooler woodland. The best modern analog is the Kibwezi Forest in Kenya, kept wet by groundwater, according to isotope expert Stanley Ambrose of the University of Illinois, Urbana-Champaign. These woods have open stands of trees, some 20 meters high, that let the sun reach shrubs and grasses on the ground.

Judging from the remains of at least 36 *Ardipithecus* individuals found so far at Aramis, this was prime feeding ground for a generalized early biped. "It was the habitat they preferred," says White.

—A.G.

note the many ways that *Ar. ramidus* differs from chimpanzees and gorillas, bolstering the argument that it was those apes that changed the most from the primitive form.

But the problem with a more "generalized model" of an arboreal ape is that "it is easier to say what it wasn't than what it was," says Ward. And if the last common ancestor, which according to genetic studies lived 5 million to 7 million years ago, didn't look like a chimp, then chimpanzees and gorillas evolved their numerous similarities independently, after gorillas diverged from the chimp/human line. "I find [that] hard to believe," says Pilbeam.

As debate over the implications of *Ar. ramidus* begins, the one thing that all can agree on is that the new papers provide a wealth of data to frame the issues for years. "No matter what side of the arguments you come down on, it's going to be food for thought for generations of graduate students," says Jungers. Or, as Walker says: "It would have been very boring if it had looked half-chimp."

—ANN GIBBONS



Past and present. *Ardipithecus*'s woodland was more like Kenya's Kibwezi Forest (left) than Aramis today.



The crawl. Researchers hunt down every fossil at Aramis.

PALEOANTHROPOLOGY

The View From Afar

How do you find priceless hominin fossils in a hostile desert? Build a strong team and obsess over the details

MIDDLE AWASH VALLEY, THE AFAR DEPRESSION, ETHIOPIA—It's about 10 a.m. on a hot morning in December, and Tim White is watching a 30-year-old farmer inch his way up a slippery hill on his knees, picking through mouse-colored rubble for a bit of gray bone. The sun is already bleaching the scrubby badlands, making it difficult to distinguish a fragment of bone in the washed-out beige and gray terrain. The only shade in this parched gully is from a small, thorny acacia tree, so the fossil hunters have draped their heads with kerchiefs that hang out from under their "Cal" and "Obama for President" baseball caps, making them look like a strange tribe of Berkeley Bedouins. If there are fossils here, White is confident that the slender farmer, Kampire Kayrento, will find them. "Kampire is the best person in the world for finding little pieces of fossilized human bone," says White, 59, a paleoanthropologist at the University of California, Berkeley, who has collected fossils in this region since 1981.

Watching Kayrento is a sort of spectator sport, because he scores so often. Just minutes earlier, he had walked over the crest of a small hill, singing softly to himself, and had spotted the fossilized core of a horn from an ancient bovid, or antelope. Then he picked up a flat piece of gray bone nearby and showed the fossil to Ethiopian paleoanthropologist Berhane Asfaw, asking, "Bovid?" Asfaw, 55, who hired Kayrento when he was a boy hanging out at fossil sites in southern Ethiopia, looked over the slightly curved piece of bone the size of a silver dollar and suggested, "Monkey?" as he handed it to White. White turned it over gently in his hands, then said: "Check that, Berhane. We just found a hominid cranium. Niiice."

As word spreads that Kayrento found a hominin, or a member of the taxon that includes humans and our ancestors, the other fossil hunters tease him: "*Homo bovid!* *Homo bovid!* Niiice."

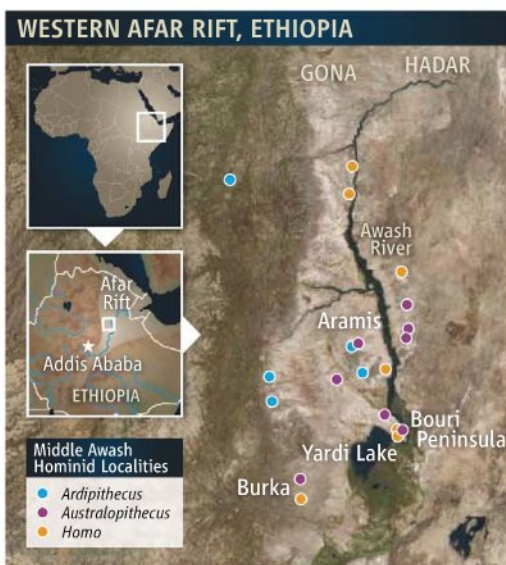
The Middle Awash project, which includes 70 scientists from 18 nations, is best known for its discovery of the 4.4-million-year-old partial skeleton of *Ardipithecus ramidus* at Aramis, about 34 kilometers north of here. That skeleton is now dramatically revising ideas

of how upright walking evolved and how our earliest ancestors differed from chimpanzees (see overview, p. 60, and main Focus text, p. 36). But Aramis is just one of 300 localities in the Middle Awash, which is the only place in the world to yield fossils that span the entire saga of hominid evolution. At last count, this team had gathered 19,000 vertebrate fossils over the past 19 years. These include about 300 specimens from seven species of hominins, from some of the first members of the human family, such as 5.8-million-year-old *Ar. ramidus kadabba*, to the earliest members of our own species, *Homo sapiens*, which lived here about 160,000 years ago.

As they work in different places in the valley, the team members travel back and forth in time. Today, this core group is working in the western foothills near the Burka catchment, where an ancient river laid down sediments 3 million to 2 million years ago and where the team has found specimens of *Australopithecus garhi*, a species they suspect may have given rise to the first members of our genus, *Homo*.

This season, after a rough start, the 25 scientists, students, cooks, and Ethiopian and Afar officials and guards in camp are working well together. Their tented camp is hours from any town, graded road, or fresh water. (They dug their own well to get water.) "The 1st week, it's like an engine that's running but not running smoothly," says White, who, with Asfaw, runs a well-organized camp where every tool, map, and shower bag has its proper place. "By the 3rd week, people know their jobs."

The 1st week, White and a paleontologist were sick, and White is still fighting a harsh cough that keeps him awake at night. The 2nd week, some aggressive Alisera tribesmen who live near the *Ar. ramidus* site threatened to kill White and Asfaw, making it difficult to return there. (That's one reason the team travels with six Afar policemen armed with AK-47s and Obama caps, dubbed "The Obama Police.") The day before, a student had awakened with a high fever and abdominal pain and had to be driven 4 hours to the nearest clinic, where he was diagnosed with a urinary tract infection, probably from drinking too little water in



Ancestral territory. The area where Ardi was found is rich in hominin fossil sites, including these worked by the Middle Awash research team.



Division of labor. Kampireo Kayrento (*top left*) homes in on fossils; he and others sweep the surface, and Giday WoldeGabriel dates sediments.

the 35°C heat. “The best laid plans change every day,” says White, who has dealt with poisonous snakes, scorpions, malarial mosquitoes, lions, hyenas, flash floods, dust tornadoes, warring tribesmen, and contaminated food and water over the years. “Nothing in the field comes easy.”

Calling the “A” team

Nothing in the Afar, for that matter, comes easy. We are reminded of that as we drive across the dusty Saragata plain to the target fossil site at 8 a.m., making giant circles in the dust with the Toyota Land Cruiser so we can find our tracks home at the end of the day. Men clad in plaid wraps, with AK-47s slung over their shoulders, flag us down seeking help. They bring over a woman who looks to be in her 70s but is probably much younger. Her finger is bleeding, and the men tell White and Asfaw, in Afar, that a puff adder bit her the night before while she was gathering wood. A quick-thinking boy had sliced her finger with a knife, releasing the venom and probably saving her life. White gets out a first-aid kit, removes a crude poultice, and cleans and bandages the wound, putting on an antibiotic cream. “It’s good she survived the night,” he says as we drive off. “The danger now is infection.”

After inching down the sandy bank of a dry river, we reach the so-called Chairman’s site. This is one of dozens of fossil localities discovered in the Burka area since 2005: exposed hillsides that were spotted in satellite and aerial photos, then laboriously explored on foot. The plan was to search for animal fossils to help date a hominid jawbone discovered last year. But in the 1st hour, with Kayrento’s discovery, they’re already on the trail of another individual instead.

As soon as White identifies the bit of skull bone, he swings into action. With his wiry frame and deep voice, he is a commanding presence, and it soon becomes clear how he earned his nickname, “The General.” In his field uniform—a suede Australian army hat with a rattlesnake band, blue jeans, and driving gloves without fingers—he uses a fossil pick to delineate the zones in the sandstone



where he wants the crew deployed. “Get everybody out of the area,” he calls to the 15 people already fanned out over the gully, scanning for fossils. “I want the ‘A’ team.” He singles out Kayrento and three others and hands them yellow pin-flags, saying, “Go back to the bottom.” As he watches them move up the slope, he warns: “Go slowly. You’re moving too fast. ... Don’t squash the slope. Move like a cat, not a cow.”

By looking at the relatively fresh fractured edge of the bone fragment, White knows

that it comes from a larger piece of skull that broke after it was exposed, not while it was buried. As Kayrento and the others find other bits of bone, they place yellow pin-flags at those spots. “This process establishes the distributional cone,” White explains. The top flag marks the highest point on the surface where the skull came out of the ground; the bottom boundary marks the farthest point where a fragment might finally have come to rest, following the fall line down the slope.

This discovery also illustrates one reason why the team comes to the field right after the rainy season. If they’re lucky, rain and floods will cut into the ancient sediments, exposing fossils. But they have to get there before the fossils disintegrate as they are exposed to the elements or are trampled by the Afar’s goats, sheep, and cattle. Timing is everything, and this season they’re a bit late. “The ideal situation is to find a fossil just as it is eroding out of the bank,” says White.

As they crawl the entire length of the gully, they turn over every rock, mud clod, and piece of carbonate rubble to make sure it doesn’t contain a fossil fragment. “Not good,” says Kayrento. “This is yucky,” agrees Asfaw, co-director of the team and the first Ethiopian scientist to join it, in 1979 when he was invited to earn his Ph.D. at Berkeley (*Science*, 29 August 2003, p. 1178).

After 2 hours, the team has collected a few more pieces of skull around the temple, forehead, and ear. “It’s getting bigger by the minute,” White says. “If we’re lucky, we’ll find it buried right in here.”

The team has to wait until the next day to find out just how lucky. At 9:45 a.m. Thursday, they return with reinforcements: Asfaw has hired two Afar men to help with the heavy lifting of buckets of dirt. With a button-down Oxford cloth shirt and a pistol stuck in the waistband of his khakis, Asfaw commands respect, and he is the best at negotiating with the Afar. In this case, he settles an argument by letting clan leaders select which men, among a large group, will get jobs.

At the site, White sets up a perimeter of blue pin-flags that look like a mini slalom course, outlining the gully that he calls the “Hot Zone” where fossil pieces are most likely to be buried. The plan is to excavate all the rock and dirt around those flags, down to the original layers of sediment. White explains that the ancient landscape would have been flatter and more verdant before tectonic movements of Earth’s crust cracked and tilted the sediment layers. But the original soil is still there, a red-brown layer of clay beneath a gray veneer of sandstone. “Throw every piece of stone out of the channel,” he orders. “If you see a hominid, I need to know right away!”

White and Kayrento literally sweep off the gray lag with a push broom and then scrape back the layers of time with a trowel to the ancient surface underneath. “Once we brush out the slopes, we’ll be

CREDITS (TOP TO BOTTOM): DAVID BRILL; TIM WHITE; DAVID BRILL

sure no fossil is left in place,” says White. In case they miss a fragment, the loose sediment is carried to giant sieves where the crew sifts it for bits of bone or teeth. The sifted rubble is taken to a circle of workers who then empty it into small aluminum pans, in which they examine every single, tiny piece—a job that gives new meaning to the word tedium. “Sieving 101,” observes Asfaw, who supervises sieving and picking today.

By 11:10 a.m., the pace of discovery has slowed. When the A team tells White it’s “not good,” he tries to infuse them with some of his energy, reminding everyone to stay focused, to keep going, to not step on fossils. But by midday, White is grumbling, too, because they’ve scoured the Hot Zone and it’s clear the skull is not there. “We’ve eliminated every hope of finding it in situ.”

Time travel

It’s a good time to take a walk with the four geologists, who are combing the terrain, hoping to find sediments with volcanic minerals to help them date the locality and its fossils precisely. While fossil hunters move slowly, stooped at the waist and focused on the ground, the geologists move fast, heads up, scanning the next horizon for a rock face with a layer cake of sediments, like those exposed in road cuts. The 6-million-year record of Middle Awash sediments is not stacked neatly in one place, with oldest rocks on the bottom and youngest on top. (If it were, the stack would be 1 kilometer thick.) Instead, the rocks are faulted and tilted into different ridges. By tracing a once-horizontal layer from ridge to ridge, sometimes for kilometers, the geologists can link the layers and place different snapshots of time into a sequence.

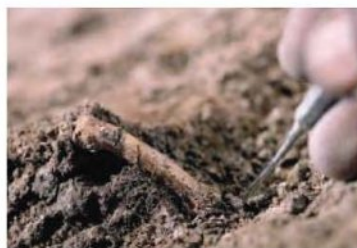
Today, Ethiopian geologist Giday WoldeGabriel of the Los Alamos National Laboratory in New Mexico, also a co-leader of the team (he joined in 1992), is searching for a familiar-looking motif—a distinct layer of volcanic tuff called the SHT (Sidiha Koma Tuff), previously dated to 3.4 million years ago by radiometric methods.

So far, the team has found just one species of hominin—*Au. garhi*—that lived at this time in the Middle Awash (*Science*, 23 April 1999, p. 629), although a more robust species, *Au. aethiopicus*, appears 2.6 million years ago in southern Ethiopia and Kenya. That’s also when the earliest stone tools appear in Gona, Ethiopia, 100 kilometers north of here. The earliest fossils of our genus *Homo* come a bit later—at 2.3 million years ago at Hadar, near Gona, also with stone tools. That’s why it is important to date *Au. garhi* precisely: Was it the maker of the stone tools left in the Afar? The team thinks *Au. garhi* is the direct descendant of the more primitive *Au. afarensis*, best known as the species that includes the famous 3.2-million-year-old skeleton of Lucy, also from Hadar. But did *Au. garhi* then evolve into early *Homo*? They need better dates—and more fossils—to find out.

“Now that we have the SHT as a reference point here, we have to try to trace it to where the new fossils are,” says WoldeGabriel. The only problem is that the SHT is several ridges and basins over from the excavation; linking the two will be difficult if not impossible. The team will also use other methods to date the new fossils.



Intensive care. Tim White uses dental tools and a gluelike adhesive to extract fragile fossils from matrix.



Luckily, the fossil hunters have found a pig known to have lived about 2.6 million to 2.7 million years ago, which suggests that the sedi-

ments and the new discovery are also that old.

At 9 a.m. Friday, 12 December, we’re back at the Chairman’s site for a 3rd day, this time with a film crew from Sweden. After White and Kayrento jokingly reenact the discovery of the skull bone for the film crew, they resume sweeping and sifting, exactly where they left off. At first, there’s little return. Berkeley postdoc Cesur Pehlevan from Ankara hands White a piece of bone: “Nope, tough luck. Right color, right thickness. Nope, sorry.”

Finally, someone hands White something special. “Oh nice, frontal bone with frontal sinus. This is getting better. That’s what we’re after,” says White. “If we can get that brow ridge, we can match it with the known species.” He turns over the new piece of frontal bone in his hand, examining it like a diamond dealer assessing a gemstone.

By the end of 3 days, the team of 20 will have collected a dozen pieces of one skull, an average yield for this region. Taken together, says White, those pieces show that “It’s an *Australopithecus* because it has a small braincase, small chewing apparatus.” There’s still not enough to identify the species, though White thinks it is *Au. garhi*. He notes that “it’s a big boy, big for an australopithecine.” If it is *Au. garhi*, that would be one more bit of evidence to suggest that *Au. afarensis* gave rise to *Au. garhi*; males are bigger than females in *Au. afarensis*—and so perhaps in *Au. garhi*, too.

For now, White and Asfaw are pleased with the new snapshot they’re getting of *Au. garhi*. On our way back to camp, White stops so we can take a photo of the moon rising over Yardi Lake in front of us, the sun setting behind us. The landscape has changed since the australopithecines were here. But one thing’s been constant in the Middle Awash, he notes: “Hominids have been right here looking at the moon rising over water for millions of years.”

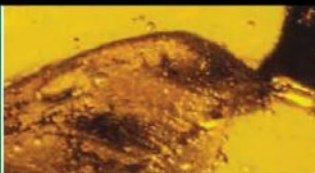
—ANN GIBBONS

“Nothing in the field comes easy.”

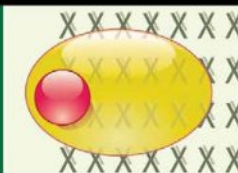
—TIM WHITE, UNIVERSITY OF CALIFORNIA, BERKELEY

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LETTERS

edited by Jennifer Sills

Think Big, Eat Small

B. WORM *ET AL.* ("REBUILDING GLOBAL FISHERIES," RESEARCH ARTICLES, 31 JULY, P. 578) REPORTED cases in which effective fisheries management was based on catch restriction, gear modification, and closed areas. Consumers can also play a role in the future of fisheries. The demand for fish continues to increase yearly—is it possible to maintain the benefits of fish consumption while minimizing the risks to both human health and global fisheries?



Sardines. Small pelagic fish such as sardines contain more nutrients and fewer contaminants than larger types of fish.

Fish at the top of the food chain can become significant repositories for a range of contaminants both natural and anthropogenic and may also have low concentrations of key nutrients. The flesh of most large predator fish from warm water fisheries (big tuna, swordfish, marlin, shark) usually is low in omega-3 fatty acids and high in mercury/selenium ratios (*1*).

Small pelagic fish, such as sardines, herrings, anchovies, and mackerel, however, have not been subject to the same overfishing pressure that has befallen almost all of the larger fish species. They not only provide higher levels of beneficial nutrients but are also significantly lower in contaminants ubiquitous to the marine food chain. They are also very affordable.

Consumers' choices are more and more influenced by health and environmental considerations. That could make a difference.

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Public Trust Doctrine: Too Limited

AS SOMEONE WHO HAS LONG ADVOCATED A coherent national ocean policy, I agree with M. Turnipseed and her colleagues that properly framed public trust concepts regarding the United States's marine environments could be an important component of federal oceans law ("Legal bedrock for

rebuilding America's ocean ecosystems," Policy Forum, 10 April, p. 183). However, the public trust doctrine—described by Turnipseed *et al.* as a "legal concept that obliges state governments to manage certain natural resources in the best interests of their citizens"—is not necessarily the "legal bedrock" that the authors portray it to be, particularly if the goal is broad-based ecosystem management.

The authors rely heavily on California's public trust doctrine, which is one of the two most expansive and ecologically protective versions of the public trust doctrines in the United States (Hawaii's is the other). Each state has its own version of the doctrine, and most have not been nearly so willing to extend their public trust law to aquatic ecosystem protection.

Indeed, as framed by the U.S. Supreme Court in the seminal case of *Illinois Central Railroad Co. v. Illinois*, 146 U.S. 387 (1892), the public trust doctrine has two main components. First, it prevents states from giving private persons control over the beds and banks of navigable waters, and hence control over the waters themselves. Thus, the resources protected under the doctrine include only bed-based natural resources such as oil and gas, gravel, and occasionally shellfish.

Second, the public trust doctrine classically preserves only three public uses of the navigable waters themselves: navigation, commerce, and fishing. This last use underscores the need to carefully construct any public trust doctrine for the United States's marine waters. Many marine fish populations are in dire trouble (*1–4*), and enshrining a right to fish in federal law would undermine, rather than promote, effective ocean ecosystem management.

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Public Trust Doctrine: Too Broad

IN THEIR POLICY FORUM ("LEGAL BEDROCK for rebuilding America's ocean ecosystems," 10 April, p. 183), M. Turnipseed *et al.* claim that extending the "public trust doctrine" to all U.S. ocean waters would more effectively promote cooperation in ocean governance than the "failing status quo." However, the authors fail to consider viable nonregulatory solutions to ocean management, such as long-term leases, second-bid

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auctions, and other public-private contractual arrangements—alternative governance mechanisms that are now commonly used to manage a wide variety of common-pool natural resources, including public lands, fisheries, and water resources (1).

In addition to conservation goals, federal ocean agencies must balance an array of competing uses of ocean resources, including energy, fishing, shipping, tourism, and military. With so many competing stakeholders in play, the public trust doctrine is too broad to provide effective guidance in ocean management. Instead of a top-down, one-size-fits-all approach, Congress should confer on U.S. ocean agencies the legal authority to experiment with alternative mechanisms to determine which solutions best promote efficiency and equity among these myriad competing uses.

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Public Trust Doctrine: In Need of Integration

THE POLICY FORUM “LEGAL BEDROCK FOR rebuilding America’s ocean ecosystems” (M. Turnipseed *et al.*, 10 April, p. 183) brings much-needed attention to ocean policy reform. The authors address the problem of too many agencies having management authority with little overall coordination. The authors’ focus on the public trust doctrine as a solution seems misplaced, however.

Most of the agencies managing resources in the Exclusive Economic Zone (EEZ) already work under a public-benefit mandate. The problem is that these agencies do not coordinate or integrate their work. It is unclear how the extension of the public trust doctrine out to the EEZ through executive order, legislation, or judicial interpretation would lead to more integrated management.

Before we introduce new laws and regulatory bodies or give existing agencies further mandates, we must research the success (or failure) of existing legislation that aims to protect the public trust. I worked for 8 years

implementing the Massachusetts regulatory program that administers the state’s Public Waterfront Act of 1866. The Act protects the public’s right in tidelands for “fishing, fowling, and navigating” and draws its legal basis from the public trust doctrine (1). Many properties within the jurisdiction of this program are not in compliance. The problem is not the lack of a legal basis but rather the limited resources allocated for compliance and enforcement with the law’s mandate (2).

To jump-start integrated management in the EEZ, we need much more than legislative, judicial, or executive backing of fundamental principles. We need regulatory mechanisms that have been proven to be effective in other comparable contexts, as well as recognition of the regional benefits of the wise use of the sea.

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Response

We welcome Craig’s support for the notion that establishing public trust doctrine principles in the United States Exclusive Economic Zone (EEZ) could prove important to federal oceans law and policy. Notwithstanding her concerns, the doctrine has burst out of its original confines—courts in many states (such as Florida, Louisiana, New Jersey, and Virginia) have expanded the doctrine’s scope to protect various natural resources and public uses, and in so doing have authorized the protection of aquatic ecosystems (1–4). Additionally, several courts have concluded that the corpus of public trusts must be preserved—not just for the benefit of the current generation, but also for future generations [e.g., (5)]. Thus, far from enshrining a right of today’s citizens to fish, applying the public trust doctrine would impose an obligation to manage fishing in federal ocean waters in a sustainable manner. Moreover, improved understanding of the interconnectedness of ocean ecosystems lends weight to the conclusion that ensuring the

ability of future generations to fish will require an ecosystem-based management regime created by means of a coastal and marine spatial planning framework (6, 7).

Guerra-Pujol asserts that we promote a public trust doctrine–based ocean policy at the expense of property rights–based management programs. However, a federal public trust doctrine would not preclude the establishment of, for example, oil, gas, and renewable energy leases and fisheries catch-share programs; instead, it would guide the development of these policies such that they protect the public interest (8).

Finally, Portman questions the added value of a federal ocean public trust doctrine when ocean-related agencies already have various mandates to act for the benefit of the U.S. public. But firmly establishing the public trust doctrine in the EEZ would explicitly impart a suite of specific duties and responsibilities to federal ocean trustees of the kind that are assumed by trustees of public, private, and charitable trusts (8, 9). The duties include those mentioned above—to preserve the trust corpus and to deal impartially among all beneficiaries (both present and future)—as well as the duties to administer the trust solely in the interest of the beneficiaries and to provide complete and accurate information to trust beneficiaries regarding the management of the trust (10).

The Massachusetts Public Waterfront Act regulatory framework has not been successful because of noncompliance and lack of enforcement. Such a circumstance should not disqualify the public trust doctrine from informing national ocean policy. Indeed, it did not prevent the Massachusetts Ocean Management Plan from identifying its impetus as the state’s public trust doctrine (11).

Would applying the public trust doctrine to the EEZ help to establish the necessary incentives, responsibilities, and powers for federal agencies to work in an integrated fashion toward long-term sustainable ocean management? We think so; by providing a common, overarching public trust mandate, as well as a suite of enforceable trusteeship duties, the doctrine would work at multiple levels to help Congress and federal agencies

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

reshape the regulatory framework used to manage U.S. ocean space and resources. It would provide the bedrock for the new national ocean policy envisioned by the president—a policy that emphasizes both inter-generational ecosystem protection and stewardship (7).

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Management for the Oceans (Island Press, Washington, DC, 2009).

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CORRECTIONS AND CLARIFICATIONS

News of the Week, ScienceInsider: "From the *Science* policy blog" (7 August, p. 665). Richard E. Besser is the former acting director of the Centers for Disease Control and Prevention. His first name is listed correctly in the online *ScienceInsider* blog.

Reports: "The C-Ala domain brings together editing and aminoacylation functions on one tRNA" by M. Guo *et al.* (7 August, p. 744). On p. 747, the citation to Fig. 4D should instead cite fig. S6.

News of the Week: "NOAA project to measure gravity aims to improve coastal monitoring" by B. Johnson (24 July, p. 378). The article incorrectly described how gravity is calculated. Gravity is determined through the difference between the measurement of an onboard gravimeter and

aircraft accelerations from GPS positioning. NASA's Gravity Recovery and Climate Experiment satellite is a source of global gravity data but not a source of vertical accelerations for the aircraft.

News Focus: "Deadly flights" by A. Curry (24 July, p. 386). The ultrasonic calls made by the bat *Nyctalus noctula* are around 22 KHz, not 32 KHz as noted in the story.

This Week in Science: "Swimming through sand" (17 July, p. 242). The credit for the image should have been "Ryan Maladen and Yang Ding; Ryan Maladen and Lionel London (inset)." The online version has been corrected.

Perspectives: "How did the turtle get its shell?" by O. Rieppel (10 July, p. 154). The photograph shows a North American snapping turtle (*Chelydra serpentina*), not a Chinese soft-shelled turtle (*Pelodiscus sinensis*) as indicated by the caption.

Reports: "Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naïve rats" by H. Vargas-Perez *et al.* (26 June, p. 1732). The second author of the paper was credited incorrectly in the author list. His name should be listed as Ryan Ting-A-Kee. The name has been corrected in the HTML version online.

Perspectives: "Extreme spinning tops" by M. Kramer (12 June, p. 1396). In the first paragraph, the rotation rate of neutrons stars was mistakenly given as up to 43,000 times per second. It should have read 43,000 times per minute.

Table of Contents: (13 March, p. 1395). In the description of the Report "Paternal control of embryonic patterning in *Arabidopsis thaliana*" by M. Bayer *et al.*, the term "cytoplasmic gene" was incorrect. The sentence should read "Transcripts of an IRAK/Pelle-like kinase gene from sperm are translated after fertilization and control asymmetric zygotic division."

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SOCIAL SCIENCES

A Plunge into Networks

David Krackhardt

The field of social networks has roots in many disciplines, including sociology, anthropology, psychology, and pure mathematics. Lately, even physicists have entered the fray. Noticeably missing from this interdisciplinary area, however, have been the economists. This is odd, in a sense, because both network analysis and economics lend themselves to rigorous mathematical modeling of social phenomena, each having a set of strong analytical tools for data analysis. On the other hand, the differences between the two fields in terms of basic assumptions about human nature are telling. Economists see the world as a set of atomistic actors, responding to incentives and fulfilling an interior goal of utility maximization. Social networkers see these same actors as mutually dependent (not atomistic), developing relationships the structure of which is itself interesting and can have profound effects on the actors' behaviors—effects that can be understood without making assumptions about an individual's psychology or utility-maximizing process. There have been previous attempts to bridge this divide (e.g., James Coleman, a sociologist who succumbed to his network of social influences at Chicago and incorporated utility maximizing in his work). But Matthew Jackson's *Social and Economic Networks* is the first concerted effort I have seen from an economist, and it is an eloquent one.

The book begins with a three-chapter introduction to key concepts within the field of social networks. The next two chapters cover random graphs and related "small world" research popularized most recently by physicists. Throughout these introductory chapters, the concept of primary interest to Jackson is centrality. Indeed, his description and development of the family of centralities are as lucid and insightful as I have seen anywhere. The book's last chapter, on "observing and measuring" networks, covers more sophisticated sociological concepts such as block modeling. Traditional network scholars will find these chapters cover familiar territory, and together the six would provide any Ph.D. student with a succinct overview of much of the social network field.

It is between the first five and final chapters

that Jackson provides a fresh perspective, using a game-theoretic approach to network questions. Chapter 6 considers the dynamics of networks: models of how they form and decay and the implications of such models. The book's next section, of four chapters, develops models of contagion, diffusion, and social influence. These include models that assume stationary network structures with actors engaging in economic behavior constrained by the networks. The section ends with a set of relatively macroeconomic considerations of network models for markets. Chapters 11 and 12 discuss more advanced methodological issues and approaches to dealing with many network-based game theory questions.

Within this vast middle, I found particularly interesting the dilemmas posed by the concepts of efficiency versus "pairwise stability." If we make some reasonable and simple assumptions about the benefits and costs that accrue to each actor as they decide which ties to form or dissolve, we find that under a wide array of conditions pairwise stability (thus an equilibrium state) is achieved without reaching an overall efficient (total-utility maximizing) state. Although this result of a stable suboptimal network is consistent with traditional sociological views of social structure, sociologists seldom appeal to utility-maximizing models to justify them. Jackson's work bridges these two perspectives by showing that even when one assumes rational behavior, suboptimal and complex states such as sociologists frequently observe often can emerge.

These rational models are instructive because they reveal the conditions under which pressures mount toward suboptimal states. I would, however, push for two extensions. Almost all of Jackson's models assume symmetric ties, yet the empirical evidence from years of traditional network research demonstrates that network ties are not typically symmetric. Some social ties will exhibit more symmetry than others, but to assume total symmetry flies in the face of reality and weakens the generality of the models.

An even more important feature of the sociological tradition in networks is the focus on the triad rather than the dyad. The

most popular engine of network dynamics is Heider's balance theory, which posits that an individual i tends to form or dissolve a tie with a prospective alter j by taking into account the affective relationship that both the individual and the alter have with a third party k . That is, whether i chooses to connect to j depends

in part on whether i and j share a positive sentiment toward k . In the small-world literature, clustering is based on this Heiderian balance. Networks are often characterized as a whole by the prevalence of all triad types (called a triad census) and which of these types are balanced. Another class of triadic predictions stems from the structural equivalence literature, which identifies roles defined as having common relations with third parties. The field is rich in triadic explanations of and evidence for network dynamics and consequences. I look forward to game theoretic models that explicitly incorporate these effects.

Social and Economic Networks

by Matthew O. Jackson

Princeton University Press,
Princeton, NJ, 2008. 518 pp. \$65,
£44.95. ISBN 9780691134406.



Romantic links. A network of relationships among high school students [from (3)].

Jackson's review of diffusion models is excellent, as tight an overview of these models as I have seen anywhere. The chapter on the related concept of social influence is also excellent, as far as it goes. However, it highlights another difference between economic and sociological thinking. For economists, the gold standard is equilibrium. For example, as Jackson underscores and the DeGroot model demonstrates, 100% agreement (consensus) is reached at equilibrium among actors in most reasonable network conditions. As a consequence, the primary analytical questions become how quickly consensus is reached and whether the consensus is "correct."

Nonetheless, we should not ignore the fact that in the real world consensus is usually not

reached. Recognizing this, most traditional social network scientists do not focus on an equilibrium of consensus. They are instead more likely to be concerned with explaining the lack of consensus (the variance) in beliefs and attitudes that appears in actual social influence contexts. Which approach is more appropriate? That is primarily a philosophy of science debate. My own belief (because I am surrounded by both economists and sociologists) is that we should not think of either as superior or more correct; rather, we learn from each perspective. In this spirit, *Social and Economic Networks* is a must-read for all those steeped in the traditional social network analysis paradigm. Economists will find Jackson offers them a superb and accessible introduction to network questions and models. And for others from any social science background curious about social networks, I recommend a careful read of the book along with Linton Freeman's historical account of the development of the field (1) and perhaps a more traditional sociological text such as Wasserman and Faust (2).

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10.1126/science.1167367

ENVIRONMENT

What Powers Have Words?

Greg Garrard

The particular genius of the greatest ornithology guides is to epitomize their subjects rather than anatomize them. It is easier, somehow, to recognize John James Audubon's birds in the field by their vividly rendered characters than to relate the living creature to a perfect rendition in three elevations. Attempting to delineate the perfect eco-poetry—the golden lines that might somehow awaken a redeeming love of nature and help us forswear our breeding, polluting, warming, murderous ways—too often, in the branch of literary study known as “ecocriticism,” produces a ghastly Platonic form answering to

all the moral or metaphysical desiderata: the crime of anthropocentrism undone, say, or the pleasure of harmony with nature extolled. John Felstiner's delightful *Can Poetry Save the Earth?* instead chooses to show us, in vivid and articulate terms, the numerable ways in which “Poems make us stop, look, and listen long enough for imagination to act, connecting, committing ourselves to the only world we've got.” Felstiner (a professor of English at Stanford University) knows as well as anyone that the obvious answer to the question in his title is “No.” Nonetheless, the rich density of his quotations and his loving attentiveness to the detail of the work of poets from William Wordsworth to Derek Walcott might persuade a sympathetic reader to, at least, a grudging “Maybe a bit.”

The book's especial distinction, among the plethora of ecocritical studies of poetry now extant, is Felstiner's determination to value poetry for its rhythms, its phonemic patterning, its semantic depth and latitude. If nothing else, he seems to suggest, poetic language in all its determined brevity and self-reference enforces an act of attention in a swirling, ever-hastening infosphere. The disruptive, alienating strangeness of a bird in a poem (Emily Dickinson's, for instance, that “glanced with rapid eyes / That hurried all around— / They looked like frightened Beads, I thought— / He stirred his Velvet Head”) might make us see a real one as if for the first time. That evergreen notion, which first occurred to a group of Russian literary theorists early in the 20th century, takes a new environmental inflection here.

Let us grant that such a perceptual shift might inspire a moral one. And let us assume that, having seen birds afresh, one might campaign for them or alter one's own lifestyle to make theirs more congenial. The merry-go-round of argument over the priority of individual and broader social change need not detain us. Nor am I persuaded that a “field guide” to the resources of environmental hope in English literature could be achieved with much more humor, insight, and interpretive tact. The larger question, unanswered as it is for the most part unaddressed in the book, is whether the values espoused by—or drawn out of—these poems can really do the duty required of them.

“Rootedness,” for instance, is supposed to flow from and mutually reinforce a deep knowledge of a particular place, like Helpston in Northamptonshire. There poet and farm laborer John Clare was so much at home that a displacement of only three

miles led him to write:

I miss the heath, its yellow furze
Molehills and rabbit tracks that lead
Through besom ling and teasel burrs
That spread a wilderness indeed

Clare's rootedness seems more pathological than worthy of emulation. He ended his days in Northampton Asylum, probably insane but possibly just accepting a welcome shelter from the contradictory demands of poetic fame, farmwork, and family life.

For American ecocritics, First Peoples supply an ideal image of rooted ecological virtue, whereas European political history has given the idea some rather alarming connotations over here. Even without the brutal joke of “blood and soil” looming (even senior Nazis referred contemptuously to their cod ecophilosophy as “Blubo”), it is far from clear what rootedness might mean to the Web

2.0 generation, many of whom care about “the environment” without knowing anything about the Earth. Above all, our students are liable to read actions at least as carefully as words and find some troubling messages there. For example, Clare aside, many of the poets in Felstiner's study seem to end up rooted in rather nice, well-appointed locations: Wordsworth moved from the poky, picturesque Dove Cottage to Rydal Mount. And Felstiner celebrates a more recent ecopoet, W. S. Merwin, who refurbished a farmhouse in the Dordogne, another in Chiapas in Mexico, and finally an abandoned pineapple plantation on Maui. Merwin's outrage about pineapple farming—“what do you think was here before the pineapple fields”—seems a little disingenuous in that light. Perhaps rootedness is merely the prize for quite old and really successful poets? Even then their livelihoods depend crucially upon a radically deracinated clientele of academics, for whom (students note) achievement is measured in terms of international recognition, not staying put.

Such cynicism is not merely corrosive. It exposes Felstiner's assumption that what we need is to love nature more. Whereas the careers of his poets suggest that there are lots of other things we might need to love to have rather less: wealth, status, babies, travel.... At the very least, a sense of reflective irony about the complex, conflicted relationship between our work as writers and our lives as citizens and consumers might deflect the most obvious of questions: If poetry were going to save the Earth, wouldn't it have done so by now?

10.1126/science.1176065

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Can Poetry Save the Earth? A Field Guide to Nature Poems

by John Felstiner

Yale University Press,
New Haven, CT, 2009. 434 pp. \$35.
ISBN 9780300137507.

POETRY

Influenced by Darwin

A session at Cambridge's Darwin Festival in early July explored connections between Darwin and current poetry. In a conversation with critic John Robert Holmes, Ruth Padel (Darwin's great-great-granddaughter), John Barnie (a Welsh writer and essayist), and Kelley Swain (a young American living in London) discussed how Darwin and his ideas have influenced their poems and how those touch on important questions that the naturalist addressed. To begin, the poets read selections from their recent works. Here we offer an example from each.

—Sherman J. Suter

10.1126/science.1181861

Fossil Memories

... or, what is left of Darwin

What is left of a man
when two hundred years have passed,
his cousins distantly pleased
with their thin-running blood,
his face on a banknote,

his home a museum
where ten children played, where he fell ill
and roused himself to walks and work countless times,
where he loved his family but lost his faith,
where he hesitated and wondered and was spooked
into writing a book which changed our future
as well as our past.

See him wrapped in cold towels
shaking with fever, or turning from his daughter's
death-bed, knowing his wife's God
would be her only solace,
or turning from his son's death-bed,
never saying aloud how nature had selected against
this loved but deficient boy.

See him hunched at a wooden table,
one hundred barnacles systematically aligned,
his touchy stomach the worse for the alcohol-preservative smell,
his eyes squinted towards the creatures he came to hate.

Weathering of time,
rust of human memory,
snowflakes of a glacier,
pebble of a mountain,
fist-sized rock of a whale's baleen,
little but a fossil of a man.

Darwin's Microscope. Kelley Swain. Flambard, Newcastle upon Tyne, UK, 2009. Paper, 72 pp. £7.50. ISBN 9781906601034.

The Question

Will nature never be ascendant again?

In between strata where you couldn't draw a breath
deep time is ticking, in pools that are poisoned and forgotten,
in deserts where nomads drive trucks shimmering like a woman's dress across salt pans,
the long seconds, the hazy hours, the mysterious years,
neither fast nor slow;
a human shakes his Mickey-Mouse watch,
Goofy wags his tail and Minnie scolds,
everything is normal and speeded up,
traffic across the city bridge a bracelet of energy,
skyscrapers hyperventilating in sunlight and rain;
the human runs then stops, runs again;
Mickey is thrown into a skip, his dippy head 'ticks' 'tocks'
with innocent, mischievous eyes;
where was I; nature's return; some mutation is
already breeding in the oceans' green reactors, some
generalist about to make good out of a bad situation;
speed the repellent we put on
to fend off extinction in the waste places
where the tap is dry above the empty bucket.

Trouble in Heaven. John Barnie. Gomer, Llandysul, Ceredigion, Wales, UK, 2007. Paper, 87 pp. £7.99, €11.27, \$11.99. ISBN 9781843237815.

A Pigeon Fancier's Manual

'This horrid Species thing.' He's got to write it new—
and smaller! Just (just!) summarizing his views.
'My rag of a book. It cost me so much labour
I almost hate it.' They build a billiard room
beside the study. He pots coloured balls like sweets
as a rest from work. She protects him from visitors
and relatives. No one's ill—they take a family holiday
on the Isle of Wight. He finishes in April.
The publisher's reader says 'Make it a manual

on pigeon-breeding! Forget the rest.
Everyone loves pigeons—it'd be reviewed
by every journal in the land.' John Murray knows
a good thing when he sees one, he'll do a small
edition but it's still too long. Edit. Vomit. Edit.
Shadows in the dawn. Resist the lure
of ever more evidence. He sends it and disappears
for a water cure. 'Severely ill.' In November,
at fifteen shillings, it sells out in one go.

Note: Quotations in the poem are from Darwin.

Darwin: A Life in Poems. Ruth Padel. Chatto and Windus, London, 2009. 160 pp. £12.99. ISBN 9780701183851. Knopf, New York, 2009. \$26. ISBN 9780307272393.

Rethinking Influenza

Rino Rappuoli,^{1*} Giuseppe Del Giudice,¹ Gary J. Nabel,² Albert D. M. E. Osterhaus,³ Robin Robinson,⁴ David Salisbury,⁵ Klaus Stöhr,⁶ John J. Treanor⁷

Today, we are better prepared to face the H1N1 influenza A 2009 virus than we were for any other previous pandemic. Although the present manufacturing capacity is unlikely to have all the vaccines needed before the peak of the next wave of cases, the potential output of vaccine manufacturing has increased from 400 to 900 million (1). A vaccine will be produced in Europe with modern cell culture technology instead of eggs. A large facility for cell culture production under construction in the United States is expected to improve the current limited production capacity. Although vaccines against avian H5N1 are not highly immunogenic, this shortcoming can be overcome by using adjuvants or reverting to using whole-virus vaccines (2–5).

Adjuvants based on oil-in-water emulsions, such as MF59 and AS03, are licensed in Europe and, although their approval in the United States is still a work in progress, they can be used under the Emergency Use Application legislation. MF59 has already been used in vaccination of more than 45 million people. Although yields may be lower than those of seasonal vaccines, preliminary data from clinical trials suggest that protection against H1N1 may be achieved with only one vaccine dose (6, 7). On 13 July, the Strategic Advisory Group of Experts (SAGE) on Immunization of the World Health Organization (WHO) advised that priority should be given to maintaining the infrastructure by vaccinating health workers, then to reducing deaths by vaccinating pregnant women and people with chronic medical conditions, followed by vaccination of children and young people to reduce transmission (8). H1N1 vaccinations should be monitored to provide information on vaccine and adjuvant safety on a large scale. It will be necessary to establish the background rates of death, miscarriages, Guillain-Barré syndrome, and so on, in popu-

lations to be vaccinated in order to see whether changes in rates occur after vaccination.

The current approach of responding to influenza outbreaks in a reactive rather than anticipatory mode is not optimal, but because most of our knowledge of influenza virus is based on data accumulated in developed countries, we have an incomplete, and sometimes inaccurate, view of virus spread and its global impacts. For example, in many low-income and tropical regions, influenza is not markedly seasonal; it persists year-round and often manifests itself as pneumonia. In the few developing countries where studies have been performed, influenza has been associated with an unexpectedly high mortality rate among infants and children (9, 10). Therefore, improved influenza surveillance in developing countries is needed, and it seems appropriate to add influenza to the vaccines recommended by the Expanded Program for Immunization (EPI). Potential sources for funding could derive from one of the existing models such as the Advanced Market Commitment. The increase in vaccination would be based on the excess manufacturing capacity for seasonal vaccines now and would encourage both international and local vaccine manufacturers to invest in additional capacity, so as to sustain the surge capacity that is necessary in case of a pandemic. Pediatric vaccination remains a problem in developed countries. At present, only the United States, Finland, and Mexico recommend vaccination of children, and implementation is low. To improve implementation, studies are required that analyze differences in seasonal patterns of the disease, whether yearly pediatric vaccination is necessary (especially in the absence of strong antigenic drift), and whether adjuvants promote multiyear protection. Vaccination during pregnancy and/or linking influenza to vaccines that target clinically important infections, such as *Streptococcus pneumoniae*, also need investigation.

Finally, a definitive solution to influenza cannot be achieved without addressing the development of additional antiviral drugs and the gaps in knowledge about the virus, including the role in protection of antibodies, T cells, and immune memory, as well as the determinants of transmissibility and disease susceptibility. Epidemiological studies need to include developing countries,

We have already learned a great deal about fighting influenza, but we need to move from a reactive to a proactive and sustainable stance.

humans, their livestock, and wild animals to be able to map the diversity and circulation of the virus.

Until H1N1, the scientific community believed that a pandemic strain could only arise from a strain that had not previously been widely disseminated in humans. However, the H1N1 virus has shown that human varieties characterized by different hemagglutinin (HA) molecules may follow separate lines of evolution and may generate potentially pandemic strains within an existing human HA type. Hence, it is essential to develop methods for estimating how many antigenically different subtypes may reside within each HA type.

Research toward development of a universal vaccine should be accelerated by testing adjuvants to increase cross-protection, conserved antigens (such as M2 and NP), or different vaccine platforms (such as the live attenuated vaccines) (11), and alternative approaches to vaccine delivery. In conclusion, although the H1N1 pandemic has the potential to cause a social and economic emergency, it also provides an opportunity to rethink our approach to influenza virus disease and to develop more effective vaccines and economically sustainable solutions for developing and developed countries.

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11. Live attenuated vaccines for seasonal vaccination are approved for immunization of individuals 2 to 49 years of age and do not cover the elderly and infants below 2 years of age.
12. The views expressed represent those of the authors, but do not necessarily reflect those of their institutions. They also do not constitute an endorsement of a specific commercial product.

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PALEONTOLOGY

Pushing Back Amber Production

David Grimaldi

Amber, the fossilized remains of tree resin, was precious and warm to Neolithic peoples, who gathered it from the cold shores of the Baltic Sea at least 13,000 years ago. Deposits of amber from Lebanon preserved insects trapped in it nearly 130 million years ago. On page 132 of this issue, Bray and Anderson (1) report that plants have been making amber for at least 320 million years. However, the most remarkable aspect of the newly discovered Carboniferous amber is that it has a molecular composition that has been seen only from angiosperms, which appeared much later in the Early Cretaceous.

Plant resins are exceedingly complex and diverse blends of compounds, and their detailed analysis is most readily achieved through the use of pyrolysis–gas chromatography–mass spectroscopy, or Py-GC-MS. Resins fall into two broad groups. Terpenoid resins are composed of isoprene units (C_5H_8), which form fused ring systems and are widespread in both conifers and angiosperms. Phenolic resins occur only in angiosperms and include lignins, flavonoids, certain pigments, the hyperallergenic coating on poison ivy leaves, and the active ingredient of marijuana (2). The readily volatile fractions of resin impart fragrance, such as the familiar scent of pine resin, but it is generally the sticky, non-volatile, di- (C_{20}) and triterpenoid (C_{30}) fractions that fossilize into amber. Resins are so diverse that those from each plant species have a distinctive Py-GC-MS fingerprint that can be used to identify the plants that produced various ambers around the world.

This fingerprint analysis allows the samples of Bray and Anderson, which were found in Illinois, to be distinguished from other plant-derived resins from the same period and to narrow down their plant origins. These other materials include Carboniferous resins, which are waxy materials, and occur mostly as resinite, or tiny granules that pepper some coals. They can also be found, rarely, as fine strands (called “resin

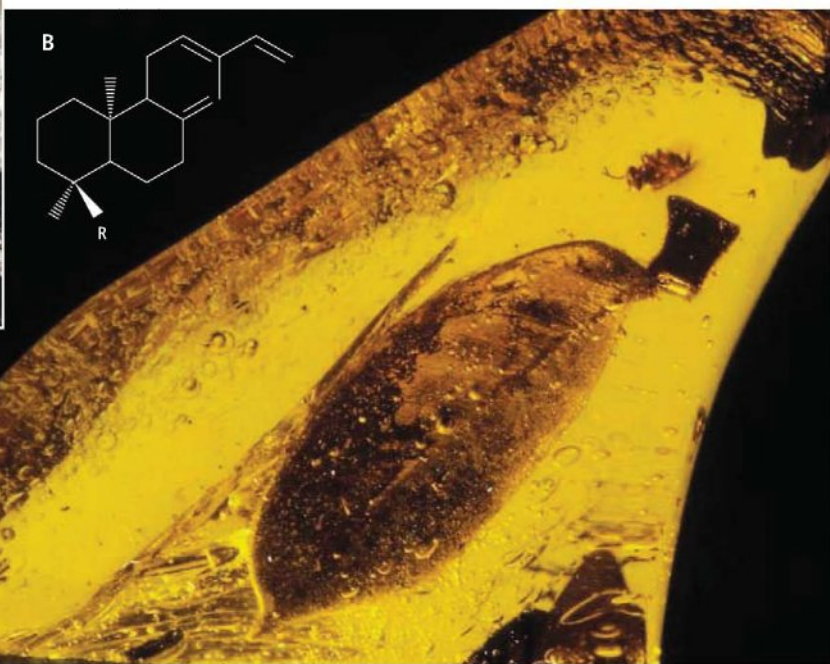
rodlets”) within the vessels of medullosan seed ferns, an extinct group that resembled modern tree ferns but that formed seeds and grew up to 10 m tall. However, it is highly unlikely that medullosans secreted the blebs of Illinois Carboniferous amber, because the few analyses of resin rodlets reveal that they are a distinctive blend of phenols and naphthalenes unlike any other resin, whether fossil or gathered from extant plants (3, 4). Other Carboniferous candidates for the Illinois amber are lycopid tree ferns (whose remains make up much of Carboniferous coals) and the extinct progymnosperms. In any case, this 320-million-year-old amber is certainly not from angiosperms, which arose almost 200 million years later. Thus, this amber casts perplexing new insight into the molecular characterization of amber.

Molecular analysis of the plethora of fossil resins has allowed their assignment into five major classes (5–7). The Illinois Carboniferous amber falls within Class I, which are polymers of labdanoid diterpenes (labdanoids

Amber from 320 million years ago is surprisingly similar to that formed much later by resins from flowering plants.

are more familiar as components of some perfumes) and their acid derivatives. In Class Ia and Ib fossil resins, communol and communol acid are the building blocks, and they have a regular configuration. Class Ia and Ib fossil resins include Baltic amber and many of the Cretaceous ambers. The Illinois Carboniferous amber is specifically a Class Ic amber, in that it has ozol and ozic acid as the building blocks in a particular stereochemical configuration called the enantio configuration (see the figure, panel B).

The best-known Class Ic ambers are from the Miocene of the Dominican Republic and Mexico (about 20 million years old) (see the figure, panel C), as well as the subfossil resins from Colombia and East Africa (only several thousand years old), also called copal. These were produced by trees in the genus *Hymenaea* (within the family Leguminosae), of which one species lives in East Africa and all others in Central and South America (4). *Hymenaea* today produces copious amounts of clear resin that thoroughly hardens. Indeed, the clarity and “carat” (specimen size) of Mexican and



Ancient ambers, inside and out. Ambers, which are fossil plant resins, have distinctive molecular structures that fall into several classes. (A) The Carboniferous amber recovered by Bray and Anderson from a coal deposit dates from 320 million years ago. It is a member of Class Ic and has a molecular framework with ozol and ozic acid building blocks. (B) The side-chain substituents in these ambers have an enantio configuration, which refers to the opposite handedness of one of the side chains on the fused rings. (C) Miocene ambers from the Dominican Republic are only 20 million years old. These are also Class Ic ambers but are derived from angiosperms. This similarity to the Carboniferous ambers is an example of convergent evolution.

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Dominican amber, as well as the particularly beautiful preservation of inclusions, makes fossils in these ambers so highly prized.

The discovery by Bray and Anderson reveals that resins of extremely similar molecular composition can be produced by entirely unrelated plants. This astonishing evolutionary convergence at the molecular level presents a cautionary message to those who study amber. It is standard practice to assign the botanical origins of an amber deposit to a genus or family of plants based just on the amber chemistry. In fact, several researchers report that nearly all Cretaceous amber was produced by the Araucariaceae, or "southern pines," a relict group of conifers that was formerly widespread and now restricted to the Southern Hemisphere (8). These claims are highly doubtful and are based almost entirely on chem-

istry, even though it is known, for example, that certain distinctive diterpene labdanoids occur in both dawn redwood (*Metasequoia*) and araucarians. This problem is one reason that future work will be needed to identify the plant that produced the Carboniferous amber from Illinois (and eventually other Paleozoic and Mesozoic amber). Such identification requires finding in situ resin within identified fossil plants.

Of the thousands of terpenoids and phenolics, most are primarily just metabolic by-products of the plant. Oozing resins serve a useful function, however, in quickly sealing a tree wound against invading fungi and insects. Detailed work by Labandeira and co-workers [reviewed in (9)] has shown that insects have chewed, sucked, galled, bored, and otherwise mined their way through Paleozoic plants. Plants may have been

defending themselves with resin far earlier than the Early Cretaceous, which is when amber becomes seriously abundant in the fossil record. Some of these questions could be answered if an insect is ever found in Carboniferous amber.

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10.1126/science.1179328

MATERIALS SCIENCE

Poison-Tolerant Fuel Cells

J. R. Selman

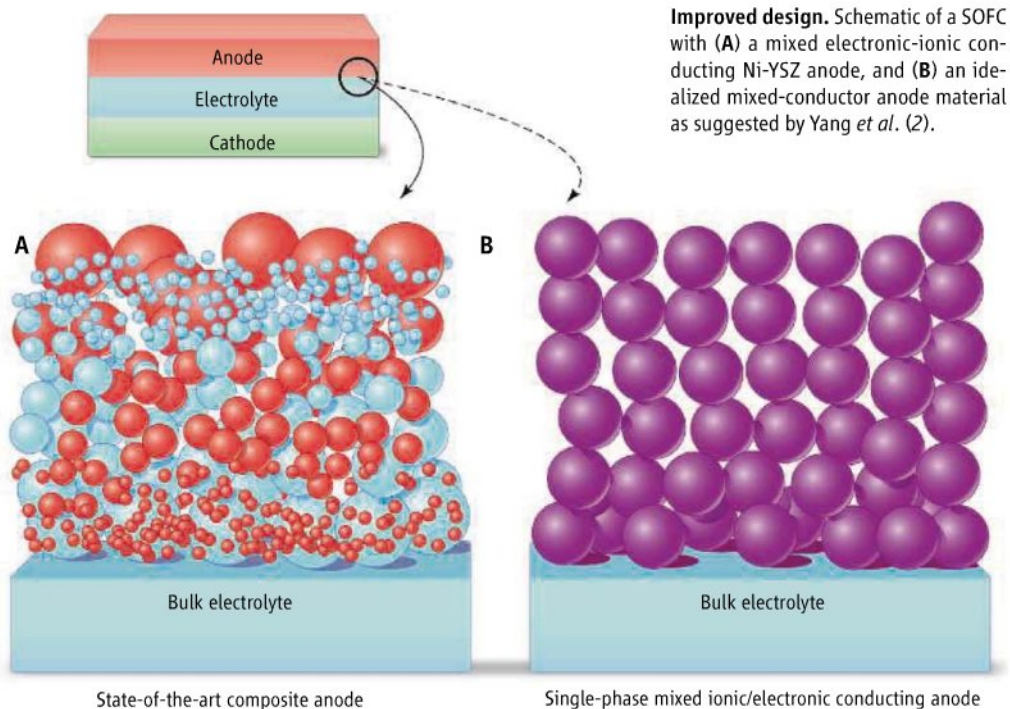
A fuel cell electrode has three functions: allow access to reacting gases, provide active electrocatalytic sites, and allow transport of electrons as well as ions (1). High-temperature fuel cells generate electrical power by oxidizing the fuel electrochemically rather than by chemical combustion. They achieve this by digesting carbon-based fuels with the help of an internal catalyst. However, sulfur in the fuel gas is a potent poison for the nickel electrocatalyst used in the anodes of such cells currently. Nickel also has another disadvantage: When CH_4 or higher hydrocarbons are used in a solid oxide fuel cell (SOFC) with a state-of-the-art nickel cermet (ceramic-metal composite) anode—that is, Ni-YSZ (yttria-stabilized zirconia)—carbon deposition, which reduces the activity of the anode, can occur if the steam-to-carbon ratio of the fuel gas is too low. Nickel effectively functions as a catalyst for carbon deposition (coking), thereby blocking the active sites of the anode and, in the worst case, destroying its structure. On page 126 of this issue, Yang *et al.*

(2) demonstrate that an alternative material can be used for the anode of the SOFC, one that provides enhanced tolerance to both poisoning and coking.

The electrochemical performance loss due to sulfur poisoning can be appreciable. For example, when just 2.5 parts per million

Alternatives to nickel-based electrocatalysts are being pursued to circumvent the problems contributing to the performance degradation of high-temperature fuel cells.

(ppm) of H_2S are added to reformed natural gas fed to a SOFC operating at 800°C , an electrochemical performance loss of 12.5% is observed (3). Loss of reforming activity is followed by the loss of anodic oxidation activity and the overall degradation of fuel cell performance. One evident cause is pro-



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gressive adsorption of sulfur on the nickel surface, which gradually blocks the active sites for anodic oxidation (3).

If the sulfur level in the reformed gas is higher than a few ppm, the poisoning may be irreversible in less than a week of operation. These observations are very similar to those in molten carbonate cells (4). But it is also known that nickel becomes less sensitive to poisoning if the fuel cell is under load. This is commonly ascribed to the oxidizing effect of H₂O product from the anodic reaction on the adsorbed sulfur. These and other observations throw doubt on a simple adsorption-dominated explanation of the poisoning mechanism (5). The microstructure of the nickel cermet anode also appears to play a role in the sensitivity of the state-of-the-art cermet to sulfur. As the vulnerability to sulfur of the Ni-YSZ cermet cannot be remedied at present, removal of sulfur to levels well below 1 ppm is required, which is possible but may add 3 to 4% to the cost of power generation.

Meanwhile, a search for alternative sulfur-resistant anode materials is ongoing. A group of leading contenders are electronically conducting oxides that can act as electrocatalysts. Such conducting oxides also are likely to be less prone to coking than nickel-containing anodes under direct reforming conditions.

Like sulfur tolerance, inhibition of coking is important not only to preserve electrode structure, but also from a system efficiency viewpoint. The mechanism of coking, like that of performance degradation due to sulfur, is not well understood at a practical level that would enable design improvements. Here, too, the role of microstructure appears to be important, and the presence of adsorbed H₂O may promote carbon nucleation.

The traditional Ni-YSZ anode is a composite mixed conductor (i.e., it conducts protons and ions simultaneously). In this case, a layered structure is used (see the figure, panel A) to inhibit electrode function while minimizing overall resistance (voltage loss due to transport and kinetics). Yang *et al.* report a different approach to anode construction. It too is based on a mixed-conductor concept, but focuses on a particular double-rare-earth doped barium zirconium cerate that exhibits mixed conduction but exists as a single phase (see the figure, panel B). This material has increased stability over a range of fuel gas compositions. The single phase could simplify anode structure considerably.

The proposed material may not offer sufficient electronic conduction to make a single-phase anode a reality. However, it has the promise of a “3-in-1 material” that resists sulfur poisoning and inhibits coking of the

anode while potentially simplifying anode structure, even if nickel or copper might have to be used as an electronic and electrocatalytic backbone. Of course, the molecular design of such a multifunctioned material would have to be quite sophisticated, as the mechanism is key in determining the effectiveness and selectivity of catalysts. Implicit in the work of Yang *et al.* is that the mixed-rare-earth doped BaZr cerate is not only a solid electrolyte but functions as a catalyst for the anodic oxidation (somewhat like the apparent function of ceria in ceria-Ni cermet). If corroborated, this would open interesting possibilities—for SOFC technology as well as hybrid high-temperature cells that could use new design strategies.

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10.1126/science.1180820

PHILOSOPHY OF SCIENCE

The Coordinates of Truth

Gary J. Nabel

The scientific method has driven conceptual inquiry for centuries and still forms the basis of scientific investigation. Yet, the hypothesis-based research paradigm itself has received scant attention recently. Here, I propose an alternative model for this paradigm, based on decision, information, and game theory. Analysis of biomedical research efforts with this model may provide a framework for predicting their likely contributions to knowledge, assessing their impact on human health, and managing research priorities.

The scientific method provides a rationale upon which scientific principles are developed, tested, and validated or rejected (1, 2). For any natural phenomenon, there is a fundamental solution or truth that explains

its basis. This solution exists in nature, regardless of whether the observer formulates the best hypothesis to explain it. It may thus be viewed as a set of coordinates in a multidimensional space: the coordinates of truth (see the first figure, panel A). By proposing hypotheses and testing their statistical validity, the hypothesis-driven experiment allows testing and validation of a scientific principle.

One goal of scientific discovery is to refine the hypothesis and increase its precision. At times, experiments yield unexpected findings and shift the view of the hypothesis without disproving it completely (see the first figure, panel B). Examples of such a paradigm shift include the discovery of reverse transcriptase (3, 4), which changed the central paradigm of biology that genetic information flowed only from DNA to RNA, and of discontinuous genes in mammalian organisms (5, 6), which revolutionized the understanding of

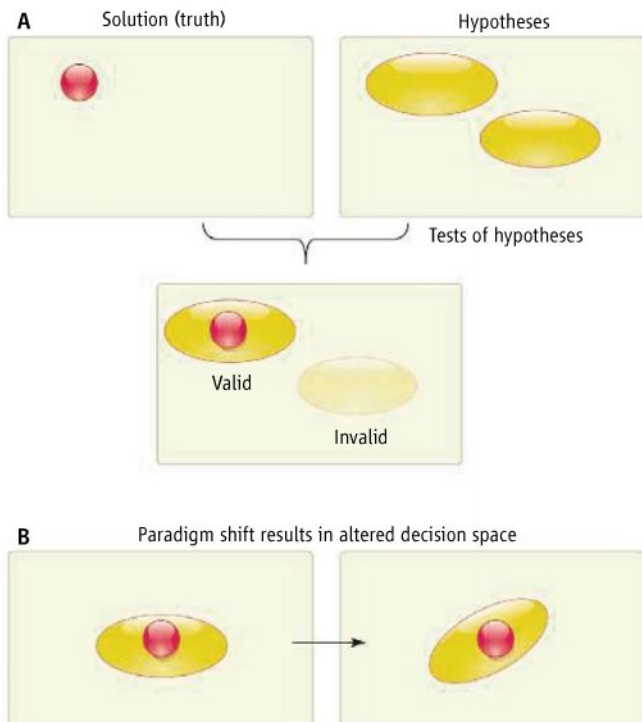
What drives scientific discovery, and how can we make it happen more effectively?

gene regulation and structure. At other times, experiments may refute a hypothesis, thereby directing attention toward more productive avenues of study.

The accuracy and predictability of a hypothesis depend on the validity of the inputs used to generate and test it. Because problems are typically complex and information regarding their solution is limited, the solution is more likely to be found if the information base is greater. This rationale is a driving force behind systems biology, which attempts to define biological complexity from a systemic perspective using information technology. Rather than testing scientific hypotheses, it provides an abundance of data that facilitates hypothesis generation.

The relative value of discovery aimed at hypothesis generation versus hypothesis testing has been debated (7, 8). High-profile journals publish systems biology studies, including the human genome sequence,

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Validating hypotheses. (A) Testing determines whether a hypothesis encompasses the true solution to the problem. (B) Experiments can lead to a change in the assumptions and predictions that encompasses the solution but fundamentally changes the decision space.

but most papers focus on hypothesis-driven investigations. Yet, there is synergy between hypothesis generation and hypothesis testing: If well designed, these efforts complement one another and can lead to fundamental breakthroughs. But how do we strike the right balance?

In the "coordinates" model, exploration through discovery research defines an unknown space with greater precision. When explored at low resolution, the solution to a problem may lie in a gap in the knowledge space. Increasing the density of information markedly raises the likelihood of defining the coordinates of a hypothesis that encompasses the solution (see the second figure).

The human genome project provides an example: Though not hypothesis-driven, it yielded a powerful information base that generated highly directed hypotheses regarding the causes of many human diseases. For scientists competing to find the best solution to a problem, game theory enters the process: Each hypothesizer games the system by using his or her own view of fragmentary

data to postulate a decision shape that contains the coordinates of the true solution. The more limited the leads, the less likely it is that the problem can be distilled to its essence. With more background, an observer is better able to constrain the variables that define the optimal solution (see the second figure).

These considerations have implications for scientific funding. For example, the investigator-initiated grants at the National Institutes of Health allow investigators to propose and test any hypothesis as long as the rationale is justified to a set of peers. The process begins with the vision of the individual scientist and ends with a judgment of its scientific merit. Recently, changes have been pro-

posed for rating these proposals, stressing their impact (9), but the evaluation remains largely subjective. The meaning of "impact" is ill defined, and there is no systematic way to assign value. In this and many other systems for awarding grants, the scientific community does not take full advantage of the scientific method to prioritize its research portfolio. For example, formal evaluation of hypotheses is not an inherent part of the review. Also, there have been few criteria by which to judge and prioritize grants for hypothesis-generating research.

How should hypothesis-generating research be evaluated? Several considerations seem relevant. If systems biology approaches are unconnected to scientific questions, they are unlikely to yield novel or fundamental insights. This research need

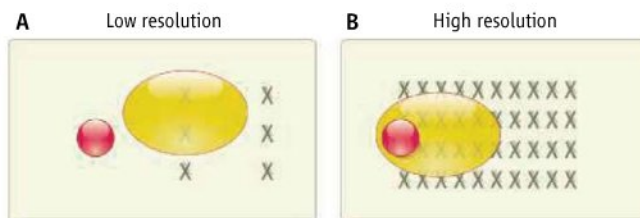
not be driven by a hypothesis, but should be directed toward a specific question. For example, transcriptional arrays have been used to understand cell transformation and characterize cancer treatment and prognosis. The usefulness of array data for addressing such questions depends on how it is collected. Analyses of tumor biopsies that contain stromal cells will be much less informative than if the RNA is derived only from tumor cells isolated, for example, by laser capture microdissection.

Although technological advances such as those of systems biology have catalyzed progress, technical innovation alone is not the solution. The value of hypothesis-generating efforts should be analyzed critically for the pertinence of the methodology to the question, the overall significance of the problem, and the likelihood of generating a viable and high-impact hypothesis. Translational research, at the nexus between clinical observation and scientific discoveries that can be applied to the treatment of human disease, may be among the best-suited applications of this approach. Reexamination of the scientific research method offers a framework not only to judge the impact of hypothesis generation on scientific discovery, but also to assess its potential to advance clinical research and treatment.

Hypothesis generation can create an organized body of knowledge from which insight can emerge. The model described here is relevant not only to biomedical research but also to other scientific disciplines. For example, in physics, the patterns of particle decay detected in the Cern Large Hadron Collider provide information that both tests and generates hypotheses about the nature of elementary particles. A modern and rigorous view of the hypothesis-driven research paradigm can similarly help to consolidate a foundation that fundamentally transforms biology and medicine.

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Generating hypotheses. Exploration of an undefined space using low-resolution (A) or high-resolution information (B) illustrates the increased likelihood of defining a hypothesis that correctly explains a complex observation.

CELL SIGNALING

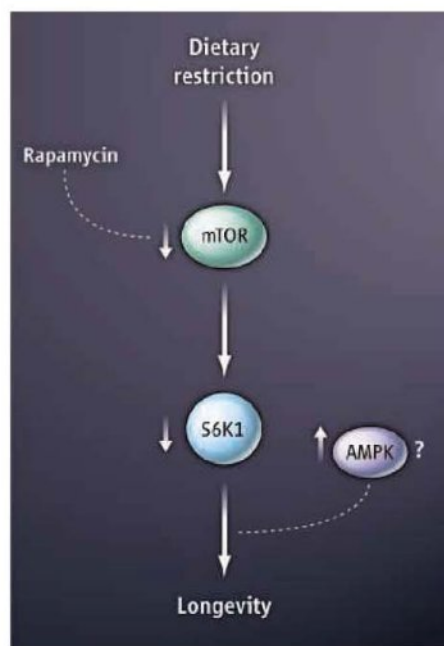
Aging Is RSKy Business

Matt Kaeberlein¹ and Pankaj Kapahi²

Following hot on the heels of recent studies showing that dietary restriction slows aging in primates (1) and that inhibiting the enzyme mammalian target of rapamycin (mTOR) increases life span in mice (2), a report by Selman *et al.* on page 140 of this issue (3) uncovers an important role for another enzyme, a ribosomal S6 protein kinase (RSK) called S6K1, as a determinant of mammalian aging. S6K1 is a target of mTOR, so the new finding further defines a conserved longevity pathway that links nutrient availability to aging in organisms as diverse as yeast and mice. The study also has potentially important implications for future attempts to increase longevity and slow the progression of age-associated diseases in humans.

RSKs phosphorylate the ribosomal protein Rps6 and consist of two subfamilies, p90^{rsk} and p70^{rsk}. S6K1 is one of two mammalian p70^{rsk} proteins that modulate mRNA translation and protein synthesis in response to mTOR signaling. Studies in yeast, nematodes, and fruit flies have shown that decreased activity of mTOR and S6K1 homologs increases life span in these species (4). Recently, the importance of mTOR in mammalian aging was demonstrated by the life span-extending effect of feeding rapamycin—a small-molecule inhibitor of mTOR—to mice (2).

Selman *et al.* suggest that the effect of rapamycin on life span likely involves reduced S6K1 activity. Consistent with this idea, phosphorylation of Rps6 decreases in rapamycin-fed mice (2). The effect of rapamycin is also greater in female than in male mice (2), which agrees with the finding of Selman *et al.* that only females lacking S6K1 (genetic “knock-out” animals) are long-lived. It is possible that inhibition of mTOR and S6K1 activity may act on life span similarly in both sexes, but that the degree of inhibition that is optimal for longevity may differ. The use of animals with different genetic backgrounds (2, 3) further complicates interpretation of the sex-dependent effects on life span, but also strengthens the idea that the mTOR-S6K1 signaling pathway is a general longevity control mechanism



Longevity pathway. A conserved signaling cascade controls life span in yeast, worms, flies, and mice.

rather than a strain-specific phenomenon.

A key finding of Selman *et al.* is the potential role for adenosine monophosphate (AMP)-activated protein kinase (AMPK) in the long life span of mice lacking S6K1. AMPK integrates energy balance with metabolism and stress resistance, and is implicated as a longevity factor in the nematode *Caenorhabditis elegans* (5). Selman *et al.* carried out microarray analysis on the long-lived mice lacking S6K1 and observed gene expression changes similar to those in animals treated with an activator of AMPK, aminoimidazole carboxamide ribonucleotide. This finding, and evidence that AMPK is activated in S6K1 knockout animals (6), suggested that life-span extension from loss of S6K1 could be mediated through increased AMPK activity. In *C. elegans*, deletion of *rsks-1* (the homolog of *S6K1*) extends life span (7). Selman *et al.* show that this effect depends on the AMPK subunit encoded by *aak-2*. Deletion of *aak-2* also suppressed the reduced body size and fecundity of *rsks-1* mutants. The potential mechanisms by which AMPK modulates the effects of *rsks-1* on both growth and longevity in *C. elegans*, and whether this function extends to mammals, have yet to be determined.

Progress in defining a signaling pathway that controls mammalian life span raises the possibility of discovering treatments for aging-related diseases.

How might AMPK activation be achieved in long-lived S6K1 knockout mice? One possibility is that mRNA coding for AMPK is preferentially translated in response to reduced S6K1 activity. Reduced mTOR-S6K1 signaling is associated with a global decrease in mRNA translation in nonmammalian species, and several additional longevity-enhancing mutations show a similar reduction in translation (8). Despite overall reduction in translation, certain mRNAs can be preferentially translated. In yeast, for example, life span-extending mutations of ribosomal proteins also result in increased translation of the mRNA coding for the Gcn4 transcription factor (9). Perhaps a similar mechanism of differential translation takes place with respect to AMPK, which could underlie subsequent activation of AMPK in animals lacking S6K1, and perhaps also in response to dietary restriction or inhibition of mTOR. The idea that AMPK activation contributes to life-span extension downstream of mTOR and S6K1 is appealing, as other studies have shown that treating mice with either of two AMPK activators, the antidiabetic drugs metformin or phenformin, modestly increases life span (10).

Although Selman *et al.* provide compelling evidence that AMPK acts downstream of S6K1 in a conserved longevity pathway, S6K1 has additional targets that could contribute to its effects on aging. For example, the hypoxia-inducible transcription factor is regulated by mTOR and is implicated in life-span extension from dietary restriction or from reduced expression of *rsks-1* in nematodes (11). Likewise, phosphorylation of Rps6 by S6K1 may be important, as mice expressing a nonphosphorylatable form of Rps6 show a decrease in cell and body size, similar to the effects of reducing the activity of S6K1 homologs in yeast, worms, and flies (12). Just as S6K1 is probably not the only mTOR target that influences aging, the story downstream of S6K1 is likely to involve more than the simple activation of AMPK.

Dietary restriction reduces signaling through the mTOR-S6K1 pathway, and this appears to be a key component of how the condition slows aging in nonmammalian organisms (4). Like dietary restriction, inhibition of mTOR or knockout of S6K1 increases life span in mice, which suggests a conserved longevity pathway that modu-

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lates aging in response to nutrient availability (see the figure). Given that dietary restriction slows aging in rhesus monkeys (1), this pathway may function similarly in primates and raises the possibility that rapamycin could mimic the antiaging effects of dietary restriction in humans (13). There are concerns, however, about potential side effects of rapamycin (most notably immune suppression) that may preclude its widespread use as a treatment for diverse aging-related diseases (13). In this regard, the study by Selman *et al.* suggests potential downstream targets of mTOR involved in mammalian aging, such as S6K1 and AMPK, that could provide longevity and health benefits without detrimental side effects when targeted pharmacologically. Although successful intervention in human aging is still an unproven proposition, an

increased understanding of the signaling pathways that modulate aging may bring us closer to this elusive goal.

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10.1126/science.1181034

ATMOSPHERE

Nitrous Oxide: No Laughing Matter

Donald J. Wuebbles

Rising atmospheric concentrations of nitrous oxide are contributing to global warming and stratospheric ozone destruction.

Despite its long-recognized importance, nitrous oxide (N_2O , also commonly referred to as laughing gas) sometimes seems like the forgotten atmospheric gas. Concerns about the stratospheric ozone layer have largely focused on reactions of ozone with chlorine and bromine atoms released from the atmospheric dissociation of chlorofluorocarbons and other anthropogenic halocarbons. Meanwhile, concerns about human-induced effects on Earth's climate have concentrated on carbon dioxide (CO_2) and methane (CH_4) emissions from fossil fuels and other sources. However, future changes in climate and in the distribution of stratospheric ozone depend on the emissions and changing atmospheric concentration of N_2O . The report by Ravishankara *et al.* on page 123 of this issue (1) not only adds to the scientific understanding of this important gas, but is also a strong reminder that nitrous oxide deserves much more attention and consideration for policy action to control future human-related emissions.

Since 1800, the atmospheric mixing ratio (that is, the concentration of N_2O relative to the

concentration of air) has increased by almost 20%, from about 270 ppb (parts per billion molecules of air) to more than 322 ppb (2, 3). In recent decades, its atmospheric concentration has been increasing at roughly 0.25% (range of 0.2 to 0.3%) per year, and this trend is set to continue. It is mainly removed from the atmosphere through photolysis and reaction with excited oxygen atoms in the middle to upper stratosphere, resulting in a long atmospheric lifetime—roughly 120 years for an e-folding (i.e., removal of 63.2% of the initial emission).

The total emissions of nitrous oxide are about 17.7 teragrams of nitrogen (Tg N) annually, but there are large uncertainty ranges on each of the individual sources. About 70% of its atmospheric emission is natural, mostly from bacterial breakdown of nitrogen in soils and in the oceans. Globally, soils in areas of natural vegetation, especially in the tropics, account for N_2O emissions of about 6.6 Tg N annually (2). Oceans account for about another 3.8 Tg N per year (2).

Human activities are responsible for the remaining 30% of N_2O emissions, or about 6.7 Tg N per year (2). The largest human-related source comes from agricultural practices and activities, including the use of syn-

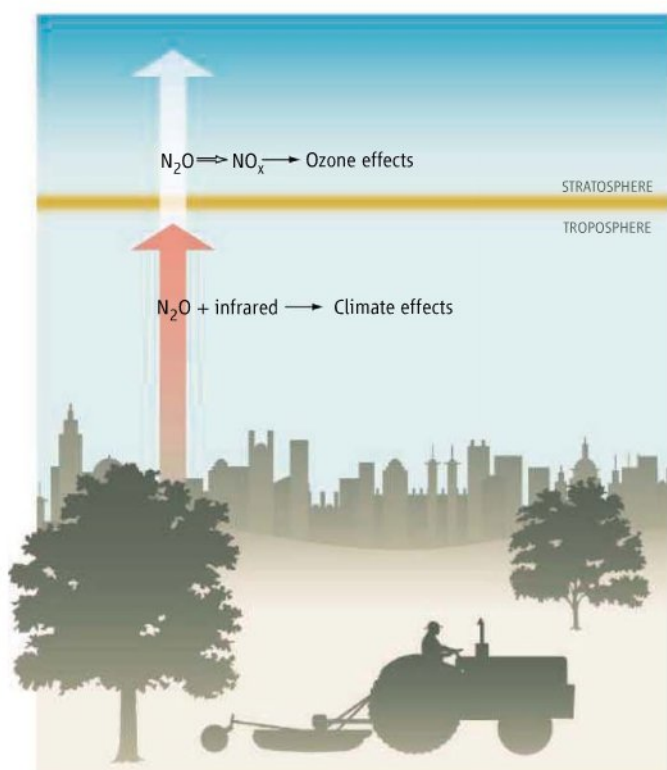
thetic and organic fertilizers, production of nitrogen-fixing crops, and application of livestock manure to croplands and pasture. These result in increased nitrogen in soils and waterways, causing N_2O emissions of about 4.5 Tg N per year (2). Nitrous oxide can also be produced during fossil fuel combustion, but the amount varies with fuel type and technology (for example, catalytic converters can produce N_2O). Fossil fuel combustion and industrial processes are responsible for N_2O emissions of around 0.7 Tg N per year (2). Other important sources include human sewage and burning of biomass and biofuels.

The vast majority of the reactive nitrogen oxides in the stratosphere result from the dissociation of nitrous oxide (through its reaction with electronically excited oxygen atoms). As a result of the high reactivity of nitrogen oxides with ozone, N_2O levels in the preindustrial atmosphere (before 1800) were sufficient to account for the majority of the natural destruction of ozone in the stratosphere. With increasing atmospheric concentration of nitrous oxide, the concentrations of nitrogen oxides in the stratosphere are also rising. Ravishankara *et al.* point out that nitrogen oxides—and, as a result, N_2O —destroy more ozone in the current stratosphere than does any other reactive chemical family.

The execution of the Montreal Protocol effectively controls the emission of some major ozone-depletion gases, particularly chlorine- and bromine-containing gases. If one assumes that only halocarbons from human activities affect ozone and that there is full global compliance with the Montreal Protocol, then the ozone layer outside the polar regions is largely expected to recover from existing human effects on the stratosphere by the middle of the 21st century (3). The Antarctic ozone “hole” is expected to take longer, until roughly 2065 (4). However, this assumes that ozone is not affected by other factors, including other human-related emissions. This assumption is false.

The increasing concentration of CO_2 in Earth's atmosphere not only warms the troposphere but also cools the stratosphere, with a tendency to increase the amount of stratospheric ozone. As a result, there is the possibility of a “super”-recovery, where the total amount of atmospheric ozone exceeds that found before 1980, before the major ozone losses due to halocarbons occurred. Furthermore, changes in climate are changing the strength of circulation patterns in the stratosphere (5), thus affecting the distribution of ozone. Potentially even more important is the continuing increase in atmospheric concentrations of nitrous oxide and methane. Meth-

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The forgotten gas. Nitrous oxide has important effects both on the climate system and on stratospheric ozone.

As a result of these effects, the distribution of ozone in the stratosphere is unlikely to resemble its pre-1980 levels at any time this century (or perhaps for several centuries to come). The total ozone column could increase but not really recover as originally expected, and it could even decrease over the course of this century, depending on what happens with these various factors. In any case, the distribution of ozone with altitude and latitude will likely remain different from the pre-1980 levels.

In addition to its effects on ozone, nitrous oxide is also the third most important gas directly affecting climate as a result of human activities. Although the increases in concentrations of CO_2 and CH_4 have been larger, N_2O is also a greenhouse gas, and its changing concentrations are important to climate change. The concerns about its effects on ozone and on climate have implications on future policies directed at nitrous

oxide. However, even if the combustion-related sources prove to be relatively easy to control, the agricultural sources may present a large challenge. Greater demand for food may affect the ability to reduce emissions from livestock and the use of fertilizers, although new approaches for increased agricultural efficiency and potential mitigation pathways continue to be developed (9).

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10.1126/science.1179571

ane affects the amount of hydrogen oxides in both the troposphere and stratosphere, which in turn affects the chemistry of ozone (6). Whereas increasing nitrous oxide will tend to destroy more ozone, increasing methane would tend to produce ozone, but each has its largest effects at different locations in the stratosphere (7, 8).

most important gas directly affecting climate as a result of human activities. Although the increases in concentrations of CO_2 and CH_4 have been larger, N_2O is also a greenhouse gas, and its changing concentrations are important to climate change. The concerns about its effects on ozone and on climate have implications on future policies directed at nitrous

CELL BIOLOGY

A Revolving Door for Calcium

Nicolas Demaurex¹ and Damon Poburko^{1,2}

Mitochondria are subcellular organelles that produce energy in the form of adenosine 5'-triphosphate (ATP) in response to chemical cues. They encode and decode cytosolic calcium (Ca^{2+}) signals, which can boost cellular respiration by regulating mitochondrial enzymes, but can also induce programmed cell death (apoptosis) by controlling the organelle's permeability. The Ca^{2+} transporters of mitochondria are well characterized functionally but have not been identified. On page 144

of this issue, Jiang *et al.* (1) show that leucine zipper EF hand-containing transmembrane protein 1 (Letm1) is one of the elusive Ca^{2+} -transport proteins. The transporter may be a good candidate for the pathogenesis of Wolf-Hirschhorn syndrome, a severe human neurological disease characterized by mental retardation and seizures.

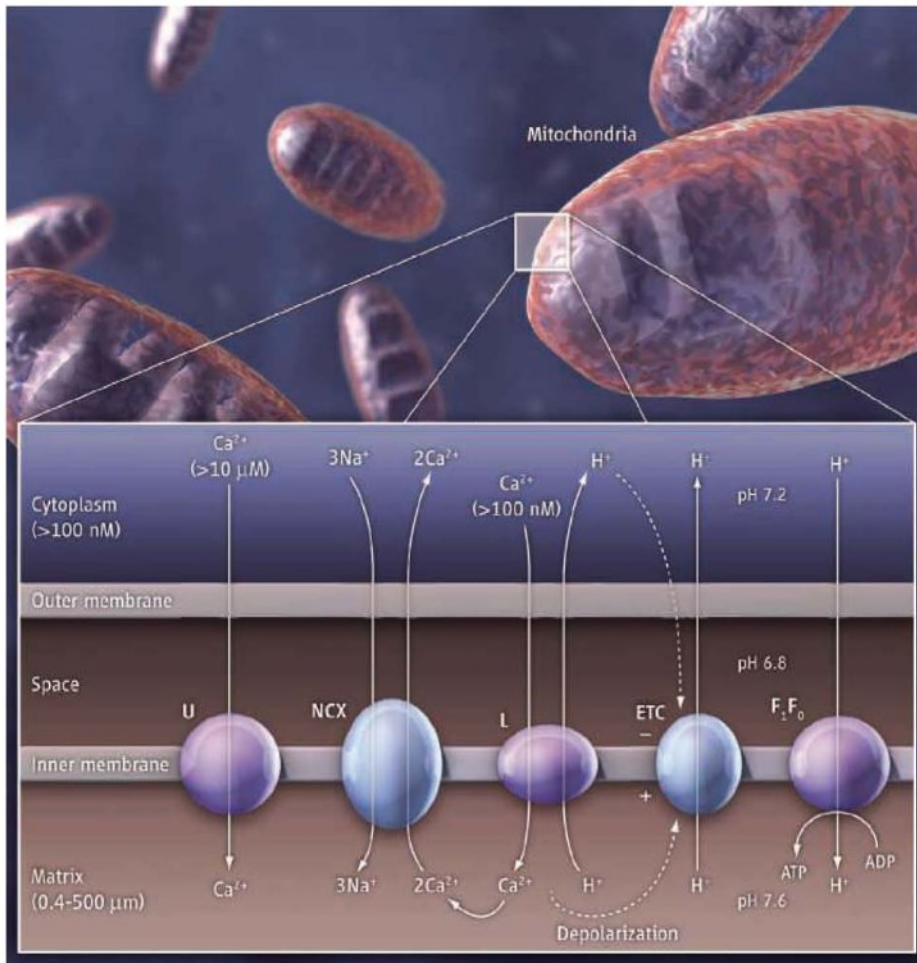
Mitochondria contain an outer membrane that is permeable to solutes, and an inner membrane that contains a Ca^{2+} uniporter, a $\text{Na}^+/\text{Ca}^{2+}$ -exchanger, and a $\text{Ca}^{2+}/\text{H}^+$ -exchanger (2). The uniporter, a Ca^{2+} -selective channel with a high capacity for Ca^{2+} uptake, mediates rapid Ca^{2+} entry driven by the negative mitochondrial potential, whereas the exchangers extrude Ca^{2+} from mitochondria (2). The uniporter operates

A mitochondrial protein linked to seizures exchanges calcium and hydrogen ions, controlling cell respiration and calcium signaling.

at micromolar concentrations of cytosolic Ca^{2+} that only occur near Ca^{2+} channels on the endoplasmic reticulum and plasma membrane (3). Mitochondria also exhibit a slower mode of Ca^{2+} uptake in response to submicromolar increases in cytosolic Ca^{2+} concentration (3). Despite decades of studies, the proteins that move Ca^{2+} across the inner membrane have remained elusive.

Functional studies of mitochondrial Ca^{2+} transporters in cells are confounded by the complex interactions between Ca^{2+} stores, plasma membrane channels, and mitochondria (4), and by the use of artificial conditions in isolated mitochondria. These limitations have led to disputed claims regarding the identity of the uniporter (5, 6). Using a fluorescent protein as a probe to measure

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Mitochondrial calcium. In mammalian mitochondria, Letm1 (L) mediates slow $\text{Ca}^{2+}/\text{H}^{+}$ exchange at low cytosolic Ca^{2+} concentrations. A uniporter (U) drives rapid Ca^{2+} entry at high cytosolic Ca^{2+} concentrations. Excess Ca^{2+} is extruded by the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX). Letm1 is bidirectional but mostly drives Ca^{2+} entry, limited by the pH gradient generated by the mitochondrial electron transport chain (ETC). The electrogenic exchange of Ca^{2+} for H^{+} depolarizes and alkalinizes mitochondria, exerting opposing effects on the proton pump (F_1F_0) that generates ATP.

mitochondrial Ca^{2+} concentration and pH simultaneously, Jiang *et al.* performed an unbiased, genome-wide RNA interference screen in cultured *Drosophila melanogaster* cells (S2 cells) to identify genes that control mitochondrial Ca^{2+} transport, and then validated the ability of candidate proteins to control ion gradients in permeabilized cultured human cells. Inhibition of *Letm1* expression by RNA interference nearly abolished both mitochondrial Ca^{2+} and pH increases, indicating that Letm1 catalyzes slow uptake of Ca^{2+} into mitochondria at submicromolar concentrations in exchange for H^{+} ions. Reconstitution of purified Letm1 in liposomes confirmed its exchange activity (one Ca^{2+} ion for one H^{+} ion) and showed Letm1 to be electrogenic.

The unusual properties of the new antiporter have several implications for cell signaling (see the figure). Ca^{2+} entry through Letm1 occurs at low Ca^{2+} concentrations

but is limited by the mitochondrial pH gradient, enabling the organelle to detect small increases in cytosolic Ca^{2+} concentration without risking Ca^{2+} overload. The 1:1 $\text{Ca}^{2+}/\text{H}^{+}$ stoichiometry, if confirmed, favors Ca^{2+} entry under most physiological Ca^{2+} concentration and pH ranges. Previous studies suggested that mitochondria possess a $1\text{Ca}^{2+}/n\text{H}^{+}$ exchanger, with $n > 2$ (2), that favors Ca^{2+} extrusion. The exchange of one divalent ion (Ca^{2+}) for a monovalent ion (H^{+}) that mitochondria use to store energy bears directly on mitochondrial bioenergetics. Electrogenic uptake of Ca^{2+} by Letm1 depolarizes mitochondria, while the concomitant extrusion of H^{+} alkalinizes mitochondria. This reduces the electrical component, but increases the chemical component, of the proton-motive force across the mitochondrial membrane that is used to generate ATP. The Letm1-depleted human cells studied by Jiang *et al.* had increased mitochondrial

potential, unlike Letm1-deficient fibroblasts from patients with Wolf-Hirschhorn syndrome (7). How Letm1 activity affects mitochondrial respiration and ATP production thus remains to be clarified.

Letm1 was previously shown to participate in mitochondrial ionic homeostasis. Yeast cells lacking Mdm38 (the homolog of Letm1) fail to swell in response to potassium acetate. This defect was rescued by the $\text{K}^{+}/\text{H}^{+}$ ionophore nigericin (8), consistent with Letm1 being a $\text{K}^{+}/\text{H}^{+}$ exchanger. However, the swelling assay requires divalent cation-free conditions, and the permeability of many Ca^{2+} transporters and channels to monovalent cations increases under such conditions. The K^{+} permeability of Letm1 at low Ca^{2+} concentrations should be clarified, because in several cell types, long-term Letm1 depletion induces mitochondrial swelling and fragmentation (7, 9). Fibroblasts from patients with Wolf-Hirschhorn syndrome have normal mitochondria, however (7, 9), indicating that morphological changes are not strictly correlated with Letm1 deficiency. Whether alterations in Ca^{2+} handling caused by Letm1 deficiency alter the morphology of mitochondria, either by disrupting their ionic homeostasis or by modifying the fusion and fission of mitochondria, is unclear.

The challenges now are to identify all mitochondrial Ca^{2+} transporters and determine their relative contribution in specific cell types. The link between Letm1 and epilepsy in patients with Wolf-Hirschhorn syndrome (10) suggests that neurons rely heavily on Letm1, but whether Letm1 deficiency alters how mitochondria handle Ca^{2+} in neurons is unknown. Understanding the function of this transporter in vivo may unravel the association between mitochondrial defects and seizures and lead to important advances in the therapy of this debilitating disease.

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11. N.D. is supported by grant 3100A0-118393 from the Swiss National Science Foundation. D.P. is a fellow of the Natural Sciences and Engineering Research Council of Canada.

10.1126/science.1180482

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INTRODUCTION

Light on the Origin of Man

CHARLES DARWIN'S SEMINAL WORK *ON THE ORIGIN OF SPECIES*, PUBLISHED 150 years ago next month, contains just one understated sentence on the implications of his theory for human evolution: "Light will be thrown on the origin of man and his history." As Darwin implied in his introduction to *The Descent of Man*, he felt that those implications were obvious; he appreciated, as events quickly showed, that it would be only natural to look at evolution foremost from our human perspective and contemplate what makes us unique among other primates—our large brains and ability to communicate, to create, and to understand and investigate our history and nature; our culture, society, and religion; the ability to run fast on two legs and manipulate tools; and more innovations that separate us from our primate relatives.

Tracing our evolution and how we came to acquire these skills and traits, however, has been difficult. Genetic data now confirm that our closest living primate relative is the chimpanzee. We shared and evolved from a common ancestor some 6 million or more years ago. But identifying our unique genes and other genetic differences between us and our primate cousins does not reveal the nature of that ancestor, nor what factors led to the genetic changes that underlie our divergent evolutionary paths. That requires a fossil record and enough parts of past species to assess key anatomical details. It also requires knowing the habitat of early humans well, to determine their diet and evaluate what factors may have influenced their evolution through time. Many early human fossils have been found, but with a few exceptions, these are all less than 4 million years old. The key first several million years of human evolution have been poorly sampled or revealed.

This issue presents 11 papers authored by a diverse international team (see following pages) describing an early hominid species, *Ardipithecus ramidus*, and its environment. The hominid fossils are 4.4 million years old, within this critical early part of human evolution, and represent 36 or more individuals, including much of the skull, pelvis, lower arms, and feet from one female. The papers represent three broad themes. Five focus on different parts of the anatomy that are revealing for human evolution. These show that *Ardipithecus* was at home both moving along trees on its palms and walking upright on the ground. Three characterize *Ardipithecus*'s habitat in detail, through analysis of the hosting rocks and thousands of fossils of small and large animals and plants. These show that *Ardipithecus* lived and ate in woodlands, not grasslands. The first paper presents an overview, and it and the last two papers trace early human evolution and synthesize a new view of our last common ancestor with chimps. One conclusion is that chimps have specialized greatly since then and thus are poor models for that ancestor and for understanding human innovations such as our ability to walk.

These papers synthesize an enormous amount of data collected and analyzed over decades by the authors. Because of the scope of these papers and the special broad interest in the topic of human evolution, we have expanded our usual format for papers and coverage. The papers include larger figures, tables, and discussions, and the overview and two concluding papers provide extended introductions and analyses.

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Ardipithecus ramidus

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*Full Research Article available online at
www.sciencemag.org/ardipithecus/

See also related video; Science Podcast

In addition, to aid understanding and introduce the main results of each paper, the authors provide a one-page summary of each paper, with an explanatory figure aimed at the general reader. Our News Focus section, written by Ann Gibbons, provides further analysis and coverage, and it includes maps and a portrait of the meticulous and at times grueling field research behind the discoveries. Available online are a video interview and a podcast with further explanations.

To accommodate this material and allow the full papers, this print issue presents an Editorial, News coverage, the authors' summaries, and four papers in full: the overview paper and one key paper from each thematic group above. The other research papers, and of course all content, are fully available online. In addition, a special online page (www.sciencemag.org/ardipithecus/) links to several print and download packages of this material for AAAS members, researchers, educators, and other readers.

This collection, essentially an extra issue of *Science* in length, reflects efforts by many behind the scenes. Every expert reviewer evaluated, and improved, multiple papers, and several commented on all 11 of them. The authors provided the summaries on top of an already large writing and revision effort. Paula Kiberstis helped in their editing. The figures and art were drafted and improved by J. H. Matternes, Henry Gilbert, Kyle Brudvik, and Josh Carlson, as well as Holly Bishop, Nathalie Cary, and Yael Kats at *Science*. Numerous other *Science* copyediting, proofreading, and production staff processed this content on top of their regular loads. Finally, special thanks go to the people of Ethiopia for supporting and facilitating this and other research into human origins over many years, and for curating *Ardipithecus ramidus* for future research and for all of us to admire.

Ardipithecus ramidus thus helps us bridge the better-known, more recent part of human evolution, which has a better fossil record, with the scarcer early human fossils and older ape fossils that precede our last common ancestor. *Ardipithecus ramidus* is a reminder of Darwin's conclusion of *The Origin*:

There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.

— BROOKS HANSON

Science

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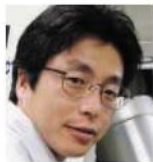
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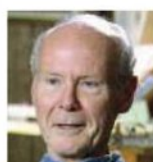
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Ardipithecus ramidus and the Paleobiology of Early Hominids

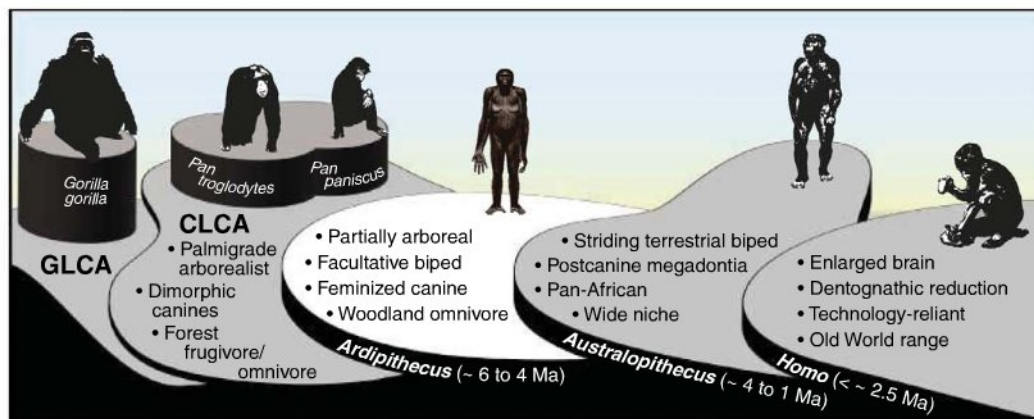
Tim D. White, Berhane Asfaw, Yonas Beyene, Yohannes Haile-Selassie, C. Owen Lovejoy, Gen Suwa, Giday WoldeGabriel

Charles Darwin and Thomas Huxley were forced to ponder human origins and evolution without a relevant fossil record. With only a few Neanderthal fossils available to supplement their limited knowledge of living apes, they speculated about how quintessentially human features such as upright walking, small canines, dexterous hands, and our special intelligence had evolved through natural selection to provide us with our complex way of life. Today we know of early *Homo* from >2.0 million years ago (Ma) and have a record of stone tools and animal butchery that reaches back to 2.6 Ma. These demonstrate just how deeply technology is embedded in our natural history.

Australopithecus, a predecessor of *Homo* that lived about 1 to 4 Ma (see figure), was discovered in South Africa in 1924. Although slow to gain acceptance as a human ancestor, it is now recognized to represent an ancestral group from which *Homo* evolved. Even after the discoveries of the partial skeleton ("Lucy") and fossilized footprints (Laetoli) of *Au. afarensis*, and other fossils that extended the antiquity of *Australopithecus* to ~3.7 Ma, the hominid fossil record before *Australopithecus* was blank. What connected the small-brained, small-canined, upright-walking *Australopithecus* to the last common ancestor that we shared with chimpanzees some time earlier than 6 Ma?

The 11 papers in this issue, representing the work of a large international team with diverse areas of expertise, describe *Ardipithecus ramidus*, a hominid species dated to 4.4 Ma, and the habitat in which it lived in the Afar Rift region of northeastern Ethiopia. This species, substantially more primitive than *Australopithecus*, resolves many uncertainties about early human evolution, including the nature of the last common ancestor that we shared with the line leading to living chimpanzees and bonobos. The *Ardipithecus* remains were recovered from a sedimentary horizon representing a short span of time (within 100 to 10,000 years). This has enabled us to assess available and preferred habitats for the early hominids by systematic and repeated sampling of the hominid-bearing strata.

By collecting and classifying thousands of vertebrate, invertebrate, and plant fossils, and characterizing the isotopic composition of soil samples and teeth, we have learned that *Ar. ramidus* was a denizen of woodland with small patches of forest. We have also learned that it



Evolution of hominids and African apes since the gorilla/chimp+human (GLCA) and chimp/human (CLCA) last common ancestors. Pedestals on the left show separate lineages leading to the extant apes (gorilla, and chimp and bonobo); text indicates key differences among adaptive plateaus occupied by the three hominid genera.

probably was more omnivorous than chimpanzees (ripe fruit specialists) and likely fed both in trees and on the ground. It apparently consumed only small amounts of open-environment resources, arguing against the idea that an inhabitation of grasslands was the driving force in the origin of upright walking.

Ar. ramidus, first described in 1994 from teeth and jaw fragments, is now represented by 110 specimens, including a partial female skeleton rescued from erosional degradation. This individual weighed about 50 kg and stood about 120 cm tall. In the context of the many other recovered individuals of this species, this suggests little body size difference between males and females. Brain size was as small as in living chimpanzees. The numerous recovered teeth and a largely complete skull show that *Ar. ramidus* had a small face and a reduced canine/premolar complex, indicative of minimal social aggression. Its hands, arms, feet, pelvis, and legs collectively reveal that it moved capably in the trees, supported on its feet and palms (palmigrade clambering), but lacked any characteristics typical of the suspension, vertical climbing, or knuckle-walking of modern gorillas and chimps. Terrestrially, it engaged in a form of bipedality more primitive than that of *Australopithecus*, and it lacked adaptation to "heavy" chewing related to open environments (seen in later *Australopithecus*). *Ar. ramidus* thus indicates that the last common ancestors of humans and African apes were not chimpanzee-like and that both hominids and extant African apes are each highly specialized, but through very different evolutionary pathways.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175802.

The Geological, Isotopic, Botanical, Invertebrate, and Lower Vertebrate Surroundings of *Ardipithecus ramidus*

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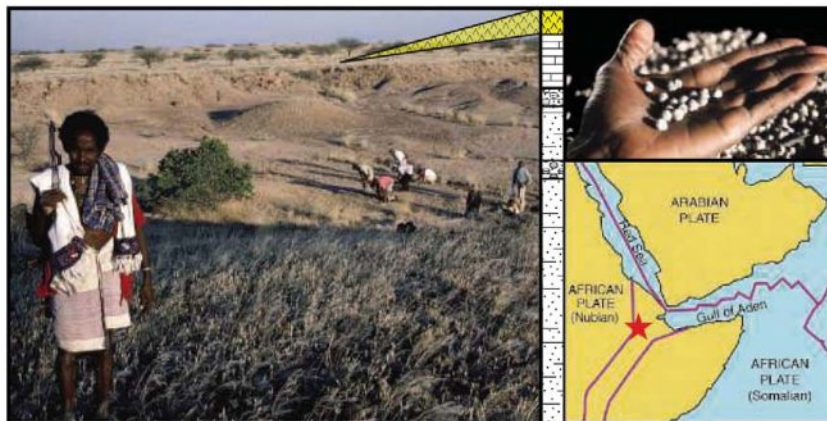
Ardipithecus ramidus was found in exposed sediments flanking the Awash River, Ethiopia. The local geology and associated fossils provide critical information about its age and habitat.

Most of Africa's surface is nondepositional and/or covered by forests. This explains why so many discoveries related to early hominid evolution have been made within eastern Africa's relatively dry, narrow, active rift system. Here the Arabian and African tectonic plates have been pulling apart for millions of years, and lakes and rivers have accumulated variably fossil-rich sediments in the Afar Triangle, which lies at the intersection of the Red Sea, Gulf of Aden, and Main Ethiopian Rifts (see map). Some of these deposits were subsequently uplifted by the rift tectonics and are now eroding. In addition, volcanoes associated with this rifting have left many widespread deposits that we can use to determine the age of these fossils using modern radioisotopic methods.

Several of the most important hominid fossils have been found near the Afar's western margin, north and west of the Awash River (star on map), including Hadar (the "Lucy" site), Gona [known for the world's oldest stone tools at 2.6 million years ago (Ma)], and the Middle Awash (including Aramis). Cumulatively, these and nearby study areas in Ethiopia have provided an unparalleled record of hominid evolution.

Fossil-bearing rocks in the Middle Awash are intermittently exposed and measure more than 1 km in thickness. Volcanic rocks near the base of this regional succession are dated to more than 6 Ma. Its uppermost sediments document the appearance of anatomically near-modern humans 155,000 years ago. As is the case for many river and lake deposits, fossil accumulation rates here have been highly variable, and the distribution and preservation of the fossils are uneven. Alterations of the fossils caused by erosion and other factors further complicate interpretation of past environments. To meet this challenge, beginning in 1981, our research team of more than 70 scientists has collected 2000 geological samples, thousands of lithic artifacts (e.g., stone tools), and tens of thousands of plant and animal fossils. The emergent picture developed from the many Middle Awash rock units and their contents represents a series of snapshots taken through time, rather than a continuous record of deposition.

Ar. ramidus was recovered from one such geological unit, 3 to 6 m thick, centered within the study area. Here, the Aramis and adjacent drainage basins expose a total thickness of 300 m of sediments largely deposited in rivers and lakes, and on floodplains, between ~5.5 and 3.8 Ma. Within this succession, the *Ar. ramidus*-bearing rock unit comprises silt and clay beds deposited on a floodplain. It is bracketed



Map showing the Middle Awash area (star) and rift locations (red lines). Photo shows the 4.4 Ma volcanic marker horizon (yellow bed) atop the locality where the skeleton and holotype teeth of *Ar. ramidus* were discovered. Also shown are some of the fossil seeds.

between two key volcanic markers, each dated to 4.4 Ma. Their similar ages and sedimentology imply that the fossils themselves date to 4.4 Ma and were all deposited within a relatively narrow time interval lasting anywhere from 100 to 10,000 years. Today the unit is exposed across a 9-km arc that represents a fortuitous transect through the ancient landscape. The western exposure, in particular, preserves a rich assemblage of plant and animal fossils and ancient soils.

Fossilized wood, seeds, and phytoliths (hard silica parts from plants) confirm the presence of hackberry, fig, and palm trees. There is no evidence of a humid closed-canopy tropical rainforest, nor of the subdesertic vegetation that characterizes the area today. Invertebrate fossils are abundant and include insect larvae, brood-balls and nests of dung beetles, diverse gastropods, and millipedes. The terrestrial gastropods best match those seen in modern groundwater forests such as the Kibwezi in Kenya. Aquatic lower vertebrates are relatively rare and probably arrived episodically during flooding of a river distal to the Aramis area. The most abundant fish is catfish, probably introduced during overbank flooding and/or by predatory birds roosting in local trees.

Our combined evidence indicates that *Ar. ramidus* did not live in the open savanna that was once envisioned to be the predominant habitat of the earliest hominids, but rather in an environment that was humid and cooler than it is today, containing habitats ranging from woodland to forest patches.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175817.

Taphonomic, Avian, and Small-Vertebrate Indicators of *Ardipithecus ramidus* Habitat

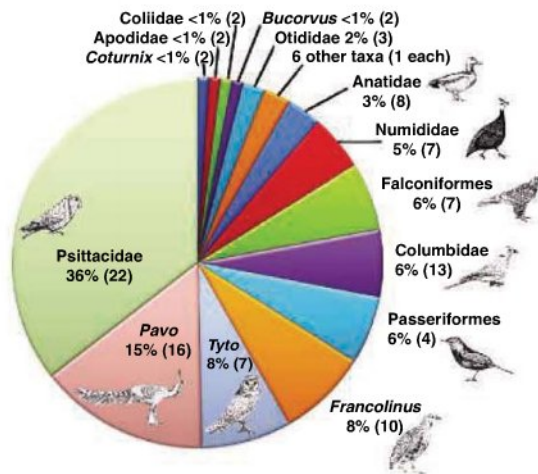
Antoine Louchart, Henry Wesselman, Robert J. Blumenschine, Leslea J. Hlusko, Jackson K. Njau, Michael T. Black, Mesfin Asnake, Tim D. White

The stratigraphic unit containing *Ardipithecus ramidus* was probably deposited rapidly, thus providing a transect through a 4.4-million-year-old landscape. To help reconstruct and understand its biological setting as thoroughly as possible, we recovered an assemblage of >150,000 plant and animal fossils. More than 6000 vertebrate specimens were identified at the family level or below. These specimens represent animals ranging in size from shrews to elephants and include abundant birds and small mammals that are usually rare in hominid-bearing assemblages. Many of these birds and small mammals are highly sensitive to environmental conditions and thus are particularly helpful in reconstructing the environment.

Accurate interpretation of fossil assemblages can be challenging. Even fossils from one layer can represent artificial amalgamations that might have originated thousands of years apart. Moreover, the remains of animals living in different habitats can be artificially mixed by flowing water or by shifting lake and river margins. Ecological fidelity can be further biased by unsystematic recovery if, for example, only the more complete, identifiable, or rare specimens are collected. Thus, interpreting the *Ardipithecus*-bearing sediments requires that we deduce the physical and biological conditions under which the fossils accumulated and the degree to which these biases operated at the time of deposition—a practice called “taphonomy.”

Both the large- and small-mammal assemblages at Aramis lack the damage that would result from transport and sorting by water, a finding consistent with the fine-grained sediments in which the bones were originally embedded. Many of the limb bone fragments of large mammals show traces of rodent gnawing and carnivore chewing at a time when the bones were still fresh. These bones were most probably damaged by hyenas, which in modern times are known to destroy most of the limb bones and consume their marrow. The actions of hyenas and other carnivores that actively competed for these remains largely explain why the fossil assemblage at Aramis contains an overrepresentation of teeth, jaws, and limb bone shaft splinters (versus skulls or limb bone ends).

As a result of this bone destruction, whole skeletons are extremely rare at Aramis, with one fortunate exception: the partial skeleton of *Ar. ramidus* excavated at ARA-VP-6/500. The relative abundance and



Abundance of birds (left) associated with *Ar. ramidus*. These distributions are consistent with a mostly woodland habitat. (Above) An example of the many small mammal and bird bones.

damage patterns of the fossils representing small mammals and birds suggest that they are derived from undigested material regurgitated by owls (owl pellets). Because of their fragility and size, bird bones have been rare or absent at most other eastern African fossil assemblages that included early hominids. However, we cataloged 370 avian fossils; these represent 29 species, several new to science. Most of the birds are terrestrial rather than aquatic, and small species such as doves, lovebirds, mousebirds, passerines, and swifts are abundant. Open-country species are rare. Eagles and hawks/kites are present, but the assemblage is dominated by parrots and the peafowl *Pavo*, an ecological indicator of wooded conditions.

The small-mammal assemblage includes up to 20 new species, including shrews, bats, rodents, hares, and carnivores. Extant counterparts live in a variety of habitats, but their relative abundance in the fossil assemblage indicates that *Ardipithecus* lived in a wooded area. Avian predators most probably procured the much rarer squirrels and gerbils from drier scrub or arid settings at a distance. Most of the bat, shrew, porcupine, and other rodent specimens are compatible with a relatively moist environmental setting, as are the abundant fossils of monkeys and spiral-horned antelopes.

The combination of geological and taphonomic evidence, the assemblage of small-mammal and avian fossils, and the taxonomic and isotopic compositions of remains from larger mammals indicate that Aramis was predominantly a woodland habitat during *Ar. ramidus* times. The anatomical and isotopic evidence of *Ar. ramidus* itself also suggests that the species was adapted to such a habitat.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175823.

Macrovertebrate Paleontology and the Pliocene Habitat of *Ardipithecus ramidus*

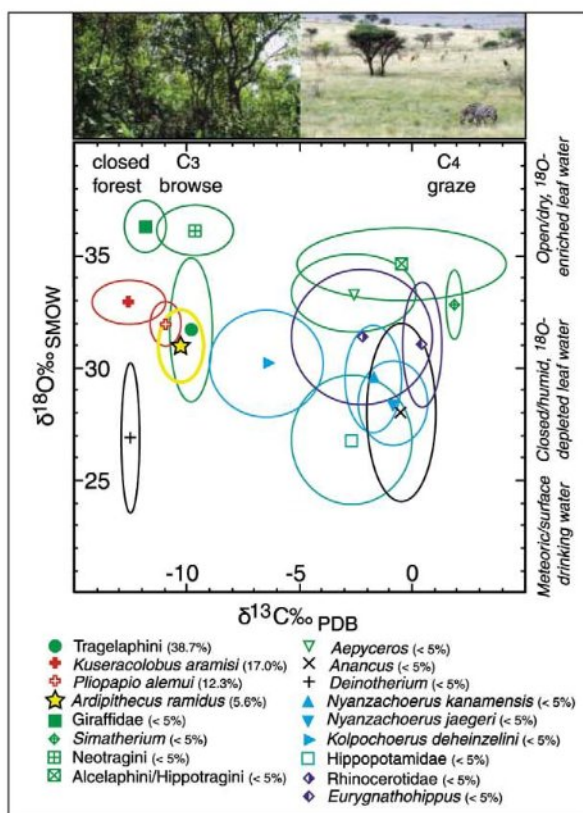
Tim D. White, Stanley H. Ambrose, Gen Suwa, Denise F. Su, David DeGusta, Raymond L. Bernor, Jean-Renaud Boisserie, Michel Brunet, Eric Delson, Stephen Frost, Nuria Garcia, Ioannis X. Giaourtsakis, Yohannes Haile-Selassie, F. Clark Howell, Thomas Lehmann, Andossa Likius, Cesur Pehlevan, Haruo Saegusa, Gina Semperebon, Mark Teaford, Elisabeth Vrba

Ever since Darwin, scholars have speculated about the role that environment may have played in human origins, evolution, and adaptation. Given that all living great apes live and feed in trees, it has been assumed that the last common ancestor we shared with these forms was also a forest dweller. In 1925, Raymond Dart described the first *Australopithecus*, a child's skull, at Taung, South Africa. Its occurrence among other fossils indicative of a grassland environment prompted speculation that the open grasslands of Africa were exploited by early hominids and were therefore somehow integrally involved with the origins of upright walking.

The *Ardipithecus*-bearing sediments at Aramis now provide fresh evidence that *Ar. ramidus* lived in a predominantly woodland setting. This and corroborative evidence from fossil assemblages of avian and small mammals imply that a grassland environment was not a major force driving evolution of the earliest hominids. A diverse assemblage of large mammals (>5 kg body weight) collected alongside *Ardipithecus* provides further support for this conclusion. Carbon isotopes from tooth enamel yield dietary information because different isotope signatures reflect different photosynthetic pathways of plants consumed during enamel development. Therefore, animals that feed on tropical open-environment grasses (or on grass-eating animals) have different isotopic compositions from those feeding on browse, seeds, or fruit from shrubs or trees. Moreover, oxygen isotopes help deduce relative humidity and evaporation in the environment.

The larger-mammal assemblage associated with *Ardipithecus* was systematically collected across a ~9 km transect of eroding sediments sandwiched between two volcanic horizons each dated to 4.4 million years ago. It consists of ~4000 cataloged specimens assigned to ~40 species in 34 genera of 16 families.

There are only three primates in this assemblage, and the rarest is *Ardipithecus*, represented by 110 specimens (a minimum of 36 individuals). Conversely, colobine monkeys and a small baboon-like monkey



Carbon and oxygen isotope analyses of teeth from the *Ar. ramidus* localities. Species are listed in order of abundance, and isotopic data separate species by what they ate and their environment.

(red crosses in figure) account for nearly a third of the entire large mammal collection. Leaf-eating colobines today exhibit strong preferences for arboreal habitats, and the carbon isotope compositions of the fossil teeth are consistent with dense to open forest arboreal feeding (see figure).

The other dominant large mammal associated with *Ar. ramidus* is the spiral-horned antelope, *Tragelaphus* (the kudu, green circle). Today, these antelopes are browsers (eating mostly leaves), and they prefer bushy to wooded habitats. The dental morphology, wear, and enamel isotopic composition of the Aramis kudu species are all consistent with such placement. In contrast, grazing antelopes (which eat mostly grass) are rare in the Aramis assemblage.

The large-mammal assemblage shows a preponderance of browsers and fruit eaters. This evidence is consistent with indications from birds, small mammals, soil isotopes, plants, and invertebrate remains. The emergent picture of the Aramis landscape during *Ar. ramidus* times is one of a woodland setting with small forest patches. This woodland graded into nearby habitats that were more open

and are devoid of fossils of *Ardipithecus* and other forest-to-woodland-community mammals. Finally, the carbon isotopic composition of *Ar. ramidus* teeth is similar to that of the predominantly arboreal, small, baboon-like *Pliopapio* and the woodland browser *Tragelaphus*, indicating little dietary intake of grass or grass-eating animals. It is therefore unlikely that *Ar. ramidus* was feeding much in open grasslands.

These data suggest that the anatomy and behavior of early hominids did not evolve in response to open savanna or mosaic settings. Rather, hominids appear to have originated and persisted within more closed, wooded habitats until the emergence of more ecologically aggressive *Australopithecus*.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175822.

The *Ardipithecus ramidus* Skull and Its Implications for Hominid Origins

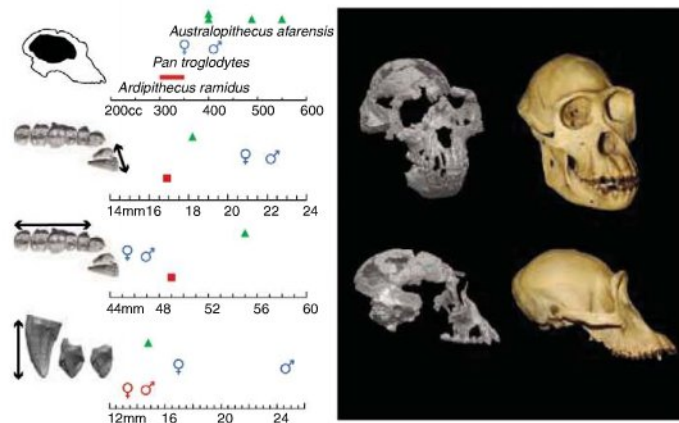
Gen Suwa, Berhane Asfaw, Reiko T. Kono, Daisuke Kubo, C. Owen Lovejoy, Tim D. White

The key feature that distinguishes *Homo sapiens* from other primates is our unusually large brain, which allows us to communicate, make tools, plan, and modify our environment. Understanding how and when our cognitive ability evolved has been a special focus in anthropology and, more recently, genetics. Fossil hominid skulls provide direct evidence of skull evolution and information about diet, appearance, and behavior. Skulls feature prominently in the characterization of species, in taxonomy, and in phylogenetic analyses of both extinct and living primates.

Unfortunately, hominid skulls are relatively rare in the fossil record. A number of partial skulls and crania (skulls without a lower jaw) of early *Homo* and its predecessor, *Australopithecus* (which lived ~1 to 4 million years ago), have been recovered, but relatively few are complete enough for extensive comparisons. One surprisingly complete but distorted cranium from 6 to 7 million years ago was discovered in central Africa (Chad). This fossil, *Sahelanthropus tchadensis* (a.k.a. "Toumaï"), is thought by many to represent the earliest known hominid, although some have argued that it is a female ape.

The *Ardipithecus ramidus* skull is of particular interest because it predates known *Australopithecus* and thereby illuminates the early evolution of the hominid skull, brain, and face. The *Ar. ramidus* skull was badly crushed, and many of its bones were scattered over a wide area. Because the bones were so fragile and damaged, we imaged them with micro-computed tomography, making more than 5000 slices. We assembled the fragments into more than 60 key virtual pieces of the braincase, face, and teeth, enough to allow us to digitally reconstruct a largely complete cranium.

The fossil skulls of *Australopithecus* indicate that its brain was ~400 to 550 cm³ in size, slightly larger than the brains of modern apes of similar body size and about a third of those of typical *Homo sapiens*. Its specialized craniofacial architecture facilitated the production of strong chewing forces along the entire row of teeth located behind its canines. These postcanine teeth were enlarged and had thick enamel, consistent with a hard/tough and abrasive diet. Some species exhibited extreme manifestations of this specialized chewing appara-



(Right) Oblique and side views of a female chimpanzee (right) and the *Ar. ramidus* female reconstruction (left; the oblique view includes a separate mandible). (Left) Comparison of brain and tooth sizes (arrows) of chimps (*Pan*; blue), *Ar. ramidus* (red), and *Australopithecus* (green). Means are plotted except for individual *Ar. ramidus* and *Au. afarensis* cranial capacities. Canine unworn heights (bottom) are based on small samples, *Ar. ramidus* (females, $n = 1$; males, $n = 3$), *Au. afarensis* ($n = 2$), *Pan* (females, $n = 19$; males, $n = 11$).



tus and are known as "robust" *Australopithecus*.

Ar. ramidus had a small brain (300 to 350 cm³), similar to that of bonobos and female chimpanzees and smaller than that of *Australopithecus*. The *Ar. ramidus* face is also small and lacks the large cheeks of "heavy chewing" *Australopithecus*. It has a projecting muzzle as in *Sahelanthropus*, which gives it a decidedly ape-like gestalt. Yet the *Ar. ramidus* skull is not particularly chimpanzee-like. For example, the ridge above the eye socket is unlike that of a chimpanzee, and its lower face does not project forward as much as a chimpanzee's face. Chimps primarily eat ripe fruits and have large incisors set in a projecting lower face. *Ar. ramidus* instead

was probably more omnivorous and fed both in trees and on the ground. Additionally, in chimpanzees, forward placement of the entire lower face is exaggerated, perhaps linked with their large tusklike canines (especially in males) and elevated levels of aggression. This is not seen in *Ar. ramidus*, implying that it was less socially aggressive.

Like *Ar. ramidus*, *S. tchadensis* had a brain that was less than 400 cm³ in size. It also resembled *Ar. ramidus* in having small non-sharpened canines. Details of the bottom of the skull show that both *Ar. ramidus* and *Sahelanthropus* had a short cranial base, a feature also shared with *Australopithecus*. Furthermore, we infer that the rear of the *Ar. ramidus* skull was downturned like that suggested for *Sahelanthropus*. These similarities confirm that *Sahelanthropus* was indeed a hominid, not an extinct ape.

These and an additional feature of the skull hint that, despite its small size, the brain of *Ar. ramidus* may have already begun to develop some aspects of later hominid-like form and function. The steep orientation of the bone on which the brain stem rests suggests that the base of the *Ar. ramidus* brain might have been more flexed than in apes. In *Australopithecus*, a flexed cranial base occurs together with expansion of the posterior parietal cortex, a part of the modern human brain involved in aspects of visual and spatial perception.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175825.

Paleobiological Implications of the *Ardipithecus ramidus* Dentition

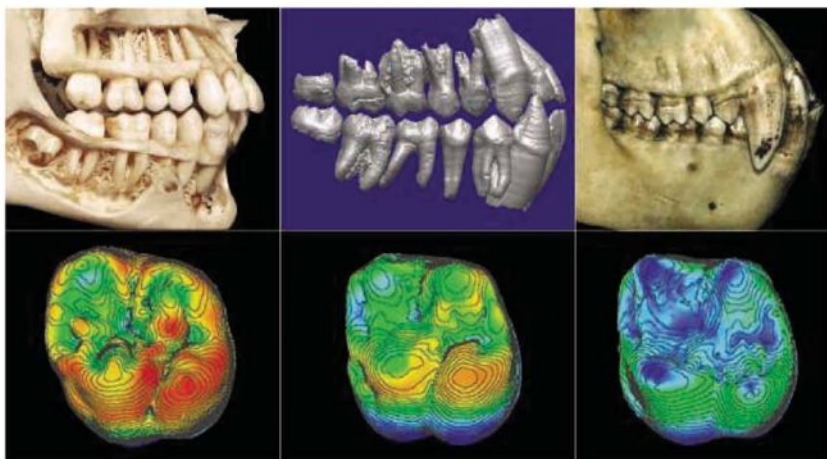
Gen Suwa, Reiko T. Kono, Scott W. Simpson, Berhane Asfaw, C. Owen Lovejoy, Tim D. White

Teeth are highly resilient to degradation and therefore are the most abundant specimens in the primate fossil record. The size, shape, enamel thickness, and isotopic composition of teeth provide a wealth of information about phylogeny, diet, and social behavior. *Ardipithecus ramidus* was originally defined in 1994 primarily on the basis of recovered teeth, but the sample size was small, limiting comparison to other primate fossils. We now have over 145 teeth, including canines from up to 21 individuals. The expanded sample now provides new information regarding *Ar. ramidus* and, using comparisons with teeth of other hominids, extant apes, and monkeys, new perspectives on early hominid evolution as well.

In apes and monkeys, the male's upper canine tooth usually bears a projecting, daggerlike crown that is continuously sharpened (honed) by wear against a specialized lower premolar tooth (together these form the C/P₃ complex). The canine tooth is used as a slicing weapon in intra- and intergroup social conflicts. Modern humans have small, stublike canines which function more like incisors.

All known modern and fossil apes have (or had) a honing C/P₃ complex. In most species, this is more developed in males than females (in a few species, females have male-like large canines, either for territorial defense or for specialized feeding). The relatively large number of *Ar. ramidus* teeth, in combination with Ethiopian *Ar. kadabba*, Kenyan *Orrorin*, and Chadian *Sahelanthropus* [currently the earliest known hominids at about 6 million years ago (Ma)], provide insight into the ancestral ape C/P₃ complex and its evolution in early hominids.

In basal dimensions, the canines of *Ar. ramidus* are roughly as large as those of female chimpanzees and male bonobos, but their crown heights are shorter (see figure). The *Ar. ramidus* sample is now large enough to assure us that males are represented. This means that male and female canines were not only similar in size, but that the male canine had been dramatically "feminized" in shape. The crown of the upper canine in *Ar. ramidus* was altered from the pointed shape seen in apes to a less-threatening diamond shape in both males and females. There is no evidence of honing. The lower canines of *Ar. ramidus* are less modified from the inferred female ape condition than the uppers. The hominid canines from about 6 Ma are similar in size to those of *Ar. ramidus*, but (especially) the older upper canines appear slightly more primitive. This suggests that male canine size and prominence were dramatically reduced by ~6 to 4.4 Ma from an ancestral ape with a honing C/P₃ complex and a moderate degree of male and female canine size difference.



Dentitions from human (left), *Ar. ramidus* (middle), and chimpanzee (right), all males. Below are corresponding samples of the maxillary first molar in each. Red, thicker enamel (~2 mm); blue, thinner enamel (~0.5 mm). Contour lines map the topography of the crown and chewing surfaces.

In modern monkeys and apes, the upper canine is important in male agonistic behavior, so its subdued shape in early hominids and *Ar. ramidus* suggests that sexual selection played a primary role in canine reduction. Thus, fundamental reproductive and social behavioral changes probably occurred in hominids long before they had enlarged brains and began to use stone tools.

Thick enamel suggests that an animal's food intake was abrasive; for example, from terrestrial feeding. Thin enamel is consistent with a diet of softer and less abrasive foods, such as arboreal ripe fruits. We measured the enamel properties of more than 30 *Ar. ramidus* teeth. Its molar enamel is intermediate in thickness between that of chimpanzees and *Australopithecus* or *Homo*. Chimpanzees have thin enamel at the chewing surface of their molars, whereas a broad concave basin flanked by spiky cusps facilitates crushing fruits and shredding leaves. *Ar. ramidus* does not share this pattern, implying a diet different from that of chimpanzees. Lack of thick enamel indicates that *Ar. ramidus* was not as adapted to heavy chewing and/or eating abrasive foods as were later *Australopithecus* or even *Homo*. The combined evidence from the isotopic content of the enamel, dental wear, and molar structure indicates that the earliest hominid diet was one of generalized omnivory and frugivory and therefore differed from that of *Australopithecus* and living African apes.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175824.

Careful Climbing in the Miocene: The Forelimbs of *Ardipithecus ramidus* and Humans Are Primitive

C. Owen Lovejoy, Scott W. Simpson, Tim D. White, Berhane Asfaw, Gen Suwa

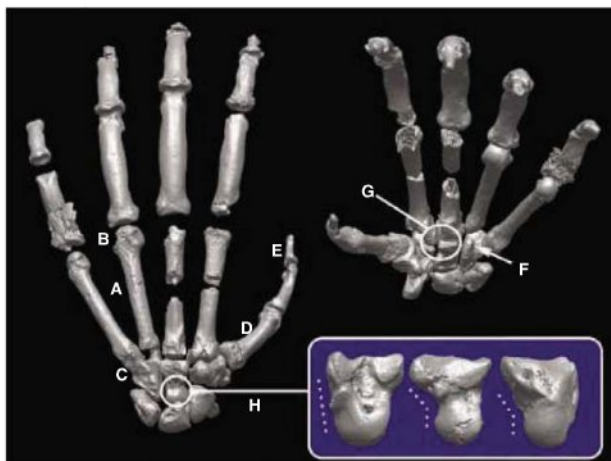
A grasping hand and highly mobile forelimb are defining characteristics of primates. The special ability to pick things up and manipulate them has probably been a central selective force in making primates so unusually intelligent. It's something that porpoises can't do at all and crows can't do very well. It may also be one reason why humans alone eventually evolved cognition.

The hands of African apes are specialized in a number of ways that make them dramatically different from our own. Apes must support their large body mass during climbing to feed and nest, especially in the middle and higher parts of the tree canopy. Their hands must therefore withstand very high forces, and this is facilitated by their elongated palms and fingers. Our palms are much shorter and our wrists more mobile. This allows us to grasp objects and compress them with great dexterity and force—something often called a “power grip.” The differences between ape and human forelimbs become less pronounced going from the hand to the shoulder. Ape and human elbow joints, for example, diverge only moderately in their manner of load transmission.

The high loads that apes bear during locomotion have required them to greatly stiffen the joints between their fingers and palms. Because their thumb has not been elongated in the same way as their palms and fingers have, thumb-to-palm and thumb-to-finger oppositions are more awkward for them. We are therefore much more adept at making and using tools. All of these forelimb characteristics in apes have led them to adopt an unusual form of terrestrial quadrupedality, in which they support themselves on their knuckles rather than on their palms. Only African apes exhibit this “knuckle-walking.” Other primates, such as monkeys, still support themselves on their palms.

It has long been assumed that our hands must have evolved from hands like those of African apes. When they are knuckle-walking, their long forelimbs angle their trunks upward. This posture has therefore long been viewed by some as “preadapting” our ancestors to holding their trunks upright.

Until now, this argument was unsettled, because we lacked an adequate fossil record. Even Lucy, the most complete *Australopithecus*



Two views of the left hand of *Ar. ramidus* showing primitive features absent in specialized apes. (A) Short metacarpals; (B) lack of knuckle-walking grooves; (C) extended joint surface on fifth digit; (D) thumb more robust than in apes; (E) insertion gable for long flexor tendon (sometimes absent in apes); (F) hamate allows palm to flex; (G) simple wrist joints; (H) capitate head promotes strong palm flexion. Inset: lateral view of capitates of *Pan*, *Ar. ramidus*, and human (left to right). Dashed lines reflect a more palmar capitate head location for *Ar. ramidus* and humans, which allows a more flexible wrist in hominids.

allowed *Ardipithecus* to support nearly all of its body weight on its palms when moving along tree branches, so that it could move well forward of a supporting forelimb without first releasing its grip on a branch.

This discovery ends years of speculation about the course of human evolution. Our ancestors' hands differed profoundly from those of living great apes, and therefore the two must have substantially differed in the ways they climbed, fed, and nested. It is African apes who have evolved so extensively since we shared our last common ancestor, not humans or our immediate hominid ancestors. Hands of the earliest hominids were less ape-like than ours and quite different from those of any living form.

Ardipithecus also shows that our ability to use and make tools did not require us to greatly modify our hands. Rather, human grasp and dexterity were long ago inherited almost directly from our last common ancestor with chimpanzees. We now know that our earliest ancestors only had to slightly enlarge their thumbs and shorten their fingers to greatly improve their dexterity for tool-using.

skeleton yet found, had only two hand bones—far short of the number needed to interpret the structure and evolution of the hand. The *Ardipithecus* skeleton reported here changes that. Not only is it more than 1 million years older than Lucy (4.4 million versus 3.2 million years old), its hands are virtually complete and intact. They show that *Ardipithecus* did not knuckle-walk like African apes and that it lacked virtually all of the specializations that protect great ape hands from injury while they climb and feed in trees.

Ardipithecus hands were very different from those of African apes. Its wrist joints were not as stiff as those of apes, and the joints between their palms and fingers were much more flexible. Moreover, a large joint in the middle of the wrist (the midcarpal joint) was especially flexible, being even more mobile than our own. This would have

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175827.

The Pelvis and Femur of *Ardipithecus ramidus*: The Emergence of Upright Walking

C. Owen Lovejoy, Gen Suwa, Linda Spurlock, Berhane Asfaw, Tim D. White

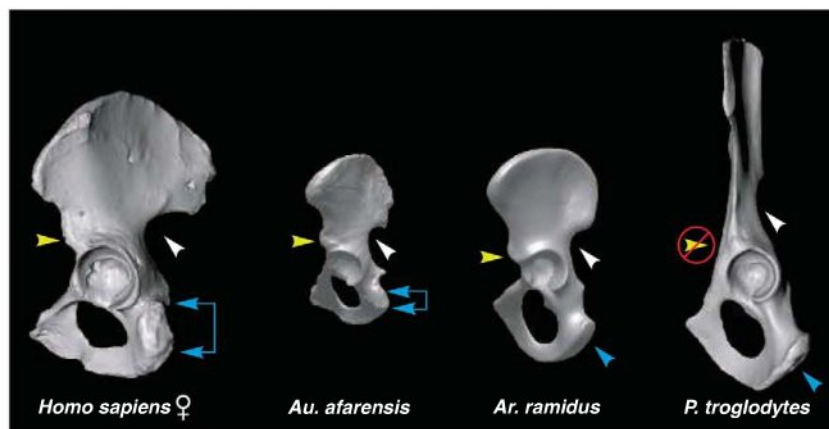
Virtually no other primate has a human-like pelvic girdle—not even our closest living relatives, the chimpanzee and bonobo. Such uniqueness evolved via substantial modifications of a pelvis more originally suited for life in trees. This arboreal primate heritage has left us rather ungainly. Our legs are massive because they continue to house almost all of the muscles originally required for climbing. Our hamstrings, the large muscles in our posterior thighs, must decelerate the swinging limb with each step, and when we run, the limb's inertia is sometimes too great and these muscles fail (not something one would want to happen on a savanna).

Furthermore, when each limb leaves the ground to be swung forward, it and the pelvis are unsupported and would slump toward the ground were it not for muscles acting on the opposite side of the body (the anterior gluteals). One early anthropologist described human locomotion as a process by which we alternately almost fall on our faces. Chimpanzees and other primates cannot prevent such slumping when walking upright because they cannot reposition these muscles effectively. Their spine is too inflexible and their ilia—the large pelvic bones to which the gluteals attach—are positioned and shaped differently than ours. Modifying a typical chimp or gorilla pelvis to facilitate upright walking would require extensive structural changes.

Until now, the fossil record has told us little about when and how the early hominid pelvis evolved. Even 3 to 4 million years ago (when our brains were still only slightly larger than those of chimpanzees), it had already undergone radical transformation. One of the oldest hominid pelvises, that of *Australopithecus afarensis* (A.L. 288-1; “Lucy”), shows that her species had already evolved virtually all of the fundamental adaptations to bipedality. Even the kinetics of her hip joint were similar to ours. Although the human pelvis was later further reshaped, this was largely the result of our much enlarged birth canal.

Ardipithecus ramidus now unveils how our skeleton became progressively modified for bipedality. Although the foot anatomy of *Ar. ramidus* shows that it was still climbing trees, on the ground it walked upright. Its pelvis is a mosaic that, although far from being chimpanzee-like, is still much more primitive than that of *Australopithecus*.

The gluteal muscles had been repositioned so that *Ar. ramidus* could walk without shifting its center of mass from side to side. This is made clear not only by the shape of its ilium, but by the appearance of a special growth site unique to hominids among all primates (the anterior inferior iliac spine). However, its lower pelvis was still



The *Ar. ramidus* pelvis has a mosaic of characters for both bipedality and climbing. Left to right: Human, *Au. afarensis* (“Lucy”), *Ar. ramidus*, *Pan* (chimpanzee). The ischial surface is angled near its midpoint to face upward in Lucy and the human (blue double arrows), showing that their hamstrings have undergone transformation for advanced bipedality, whereas they are primitive in the chimpanzee and *Ar. ramidus* (blue arrows). All three hominid ilia are vertically short and horizontally broad, forming a greater sciatic notch (white arrows) that is absent in *Pan*. A novel growth site [the anterior inferior iliac spine (yellow arrows)] is also lacking in *Pan*.

almost entirely ape-like, presumably because it still had massive hindlimb muscles for active climbing.

Changes made in the upper pelvis rendered *Ar. ramidus* an effective upright walker. It could also run, but probably with less speed and efficiency than humans. Running would also have exposed it to injury because it lacked advanced mechanisms such as those that would allow it to decelerate its limbs or modulate collision forces at its heel. *Australopithecus*, which had given up its grasping foot and abandoned active climbing, had evolved a lower pelvis that allowed it to run and walk for considerable distances.

Ar. ramidus thus illuminates two critical adaptive transitions in human evolution. In the first, from the human-chimp last common ancestor to *Ardipithecus*, modifications produced a mosaic pelvis that was useful for both climbing and upright walking. In the second, from *Ardipithecus* to *Australopithecus*, modifications produced a pelvis and lower limb that facilitated more effective upright walking and running but that were no longer useful for climbing. Because climbing to feed, nest, and escape predators is vital to all nonhuman primates, both of these transitions would likely have been a response to intense natural selection.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175831.

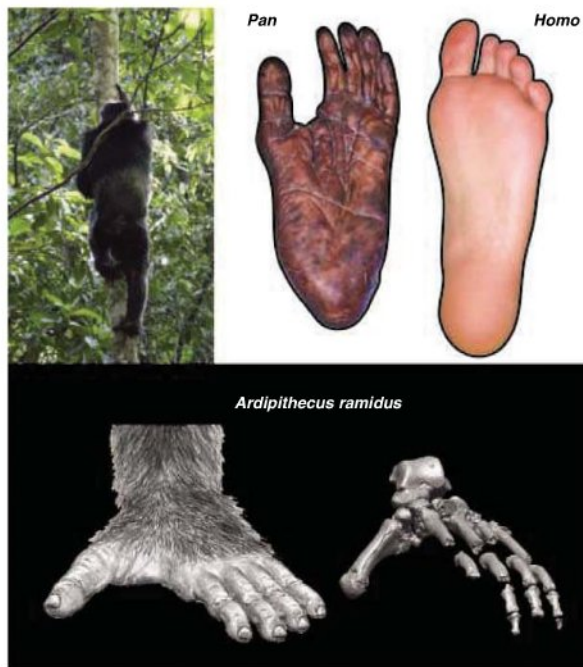
Combining Prehension and Propulsion: The Foot of *Ardipithecus ramidus*

C. Owen Lovejoy, Bruce Latimer, Gen Suwa, Berhane Asfaw, Tim D. White

The special foot adaptations that enable humans to walk upright and run are central to understanding our evolution. Until the discovery of *Ardipithecus ramidus*, it was generally thought that our foot evolved from one similar to that of modern African apes. Apes have feet that are modified to support their large bodies and to facilitate vertical climbing, thus allowing them to feed, nest, and seek safety in trees. Our foot differs from theirs in myriad ways, and its evolution from theirs would consequently have required an extensive series of structural changes. Some mid-20th-century comparative anatomists were so impressed with the profound differences between human and extant ape feet that they postulated a deep, pre-ape origin for hominids.

Ar. ramidus brings a new perspective to this old controversy. Its foot turns out to be unlike those of the African apes in many ways. The partial skeleton of *Ar. ramidus* preserves most of the foot and includes a special bone called the os peroneum that is critical for understanding foot evolution. This bone, which is embedded within a tendon, facilitates the mechanical action of the fibularis longus, the primary muscle that draws in the big toe when the foot is grasping. Until now, we knew little about this bone's natural history, except that it is present in Old World monkeys and gibbons but generally not in our more recent ape relatives. Monkeys are very accomplished at leaping between trees. They must keep their feet fairly rigid during takeoff when they hurl themselves across gaps in the tree canopy; otherwise, much of the torque from their foot muscles would be dissipated within the foot rather than being transferred to the tree.

The African apes are too large to do much leaping. They have therefore given up the features that maintain a rigid foot and have instead modified theirs for more effective grasping—almost to the point of making it difficult to distinguish their feet from their hands. Indeed, very early anatomists argued that the “quadrumanus” apes were not related to humans because of their hand-like feet. Extant apes lack the os peroneum, and their fibularis tendon, which draws



Foot skeleton of *Ar. ramidus* (bottom; reconstruction based on computed tomography rendering shown) lacked many features that have evolved for advanced vertical climbing and suspension in extant chimpanzees (*Pan*, top left). Chimpanzees have a highly flexible midfoot and other adaptations that improve their ability to grasp substrates. These are absent in *Ar. ramidus*.

the great toe closed during grasping, has been relocated more toward the front of the foot. This makes the tendon run more parallel to other joints that cross the midfoot, and allows apes to grasp with great power without stiffening these other, flexible joints. Apes can thus both powerfully grasp and mold their feet around objects at the same time. However, their feet have become less effective as levers, making them far less useful in terrestrial propulsion.

The foot of *Ar. ramidus* shows that none of these ape-like changes were present in the last common ancestor of African apes and humans. That ancestor, which until now has been thought to be chimpanzee-like, must have had a more monkey-like foot. Not only did it still have an os peroneum, it must also have had all of the other characteristics associated with it (subsequently abandoned in chimpanzees and gorillas). We infer this because humans still have these characteristics, so we must have retained them from our last common ancestor. The mid-20th-century anatomists were correct to worry about the human

foot as they did: Ours turns out to have evolved in one direction, while those of African apes were evolving in quite another.

One of the great advantages of our more rigid foot is that it works much better as a lever during upright walking and running (as it also does in monkeys). However, *Ar. ramidus* still had an opposable big toe, unlike any later hominid. Its ability to walk upright was therefore comparatively primitive. Because it had substantially modified the other four toes for upright walking, even while retaining its grasping big toe, the *Ardipithecus* foot was an odd mosaic that worked for both upright walking and climbing in trees. If our last common ancestor with the chimpanzee had not retained such an unspecialized foot, perhaps upright walking might never have evolved in the first place.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175832.

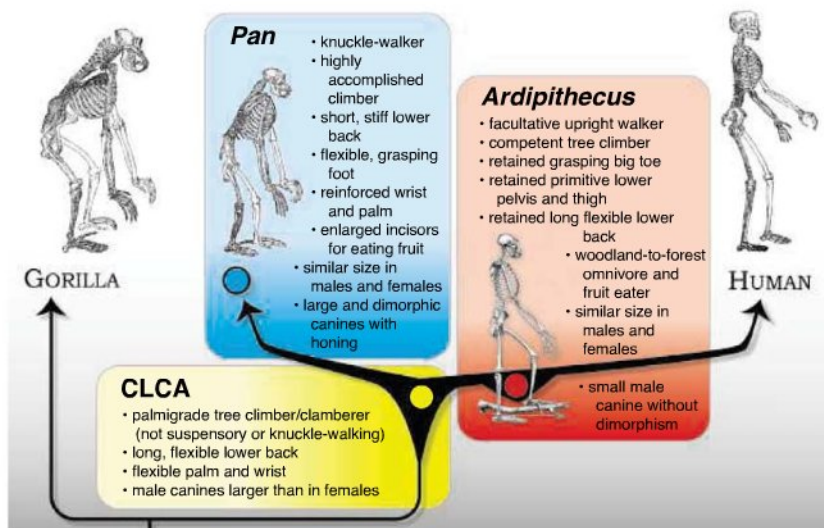
The Great Divides: *Ardipithecus ramidus* Reveals the Postcrania of Our Last Common Ancestors with African Apes

C. Owen Lovejoy, Gen Suwa, Scott W. Simpson, Jay H. Matternes, Tim D. White

Evolutionary biologists have long recognized that the living primates most similar to humans are the great apes, and comparative genomic sequence analyses confirm that we are most closely related to chimpanzees and bonobos (genus *Pan*). Because of our great genomic similarity (sometimes even cited as ~99%), the presumption that we evolved from a chimpanzee-like ancestor has become increasingly common wisdom. The widely held view that the genomic and phyletic split between *Pan* and humans was as recent as 5 to 6 million years ago also fuels the often uncritical acceptance of a *Pan*-like last common ancestor. *Ardipithecus ramidus* at 4.4 million years ago provides the first substantial body of fossil evidence that temporally and anatomically extends our knowledge of what the last common ancestor we shared with chimpanzees was like, and therefore allows a test of such presumptions.

Until now, *Australopithecus afarensis*, which lived 3 to 4 million years ago, represented the most primitive well-known stage of human evolution. It had a brain only slightly larger than that of chimpanzees, and a snout that projected more than in later hominids. Assuming some variant of a chimpanzee-like ape ancestry, the bipedality of *Au. afarensis* has been widely interpreted as being so primitive that it probably could not have extended either its hip or knee joints and was a clumsy upright walker. Some researchers have even postulated that *Au. afarensis* could walk but not run, or vice versa. Still others have suggested that *Au. afarensis* had a grasping ape-like foot. Similarly, it has been suggested that *Au. afarensis* had forelimbs that were ape-like, including long, curved fingers used to forage daily in the arboreal canopy, and that its immediate ancestors must have knuckle-walked. *Australopithecus* males were noticeably larger than females, and this has often been interpreted as signifying a single-male, polygynous, *Gorilla*-like mating system. Unlike gorillas, it has diminutive canines, but these were argued to be a consequence of its huge postcanine teeth. Early hominids have even been posited to have possibly interbred with chimpanzees until just before the appearance of *Australopithecus* in the fossil record.

The *Ar. ramidus* fossils and information on its habitat now reveal that many of these earlier hypotheses about our last common ancestor with chimpanzees are incorrect. The picture emerging from *Ar. ramidus* is that this last common ancestor had limb proportions more like those of monkeys than apes. Its feet functioned only partly like those of apes and much more like those of living monkeys and early



Cladogram adding *Ar. ramidus* to images of gorilla, chimpanzee, and human, taken from the frontispiece of *Evidence as to Man's Place in Nature*, by Thomas H. Huxley (London, 1863) (with the positions of *Gorilla* and *Pan* reversed to reflect current genetic data). Numerous details of the *Ar. ramidus* skeleton confirm that extant African apes do not much resemble our last common ancestor(s) with them.

apes such as *Proconsul* (which lived more than 15 million years ago). Its lower back was mobile and probably had six lumbar vertebrae rather than the three to four seen in the stiff backs of African apes. Its hand was unpredictably unique: Not only was its thumb musculature robust, unlike that of an ape, but its midcarpal joint (in the wrist) allowed the wrist to bend backward to a great degree, enhancing its ability to move along tree branches on its palms. None of the changes that apes have evolved to stiffen their hands for suspension and vertical climbing were present, so its locomotion did not resemble that of any living ape.

The hominid descendant of the last common ancestor we shared with chimpanzees (the CLCA), *Ardipithecus*, became a biped by modifying its upper pelvis without abandoning its grasping big toe. It was therefore an unpredicted and odd mosaic. It appears, unlike *Au. afarensis*, to have occupied the basal adaptive plateau of hominid natural history. It is so rife with anatomical surprises that no one could have imagined it without direct fossil evidence.

See pages 62–63 for authors' affiliations.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175833.

Reexamining Human Origins in Light of *Ardipithecus ramidus*

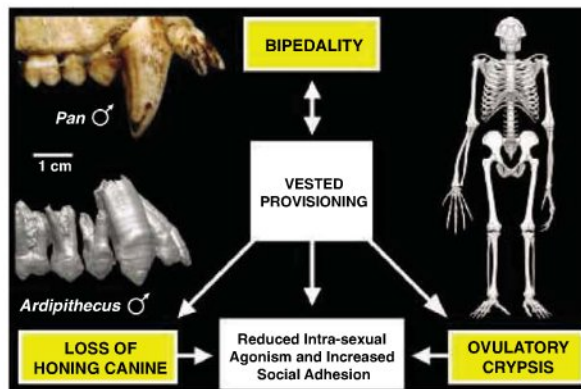
C. Owen Lovejoy

Chimpanzees, bonobos, and gorillas are our closest living relatives. The most popular reconstructions of human evolution during the past century rested on the presumption that the behaviors of the earliest hominids were related to (or even natural amplifications of) behaviors observed in these living great apes. One effect of chimpanzee-centric models of human evolution has been a tendency to view *Australopithecus* as transitional between an ape-like ancestor and early *Homo*.

Ardipithecus ramidus nullifies these presumptions, as it shows that the anatomy of living African apes is not primitive but instead has evolved specifically within extant ape lineages. The anatomy and behavior of early hominids are therefore unlikely to represent simple amplifications of those shared with modern apes. Instead, *Ar. ramidus* preserves some of the ancestral characteristics of the last common ancestor with much greater fidelity than do living African apes. Two obvious exceptions are its ability to walk upright and the absence of the large projecting canine tooth in males, derived features that *Ardipithecus* shares with all later hominids.

Ar. ramidus illuminates our own origins because it clarifies our relationship to *Australopithecus*. For example, the enlarged rear teeth of *Australopithecus* have long been viewed as adaptations to a rough, abrasive diet. This has led to speculation that canine teeth might have become smaller simply to accommodate the emergence of these other enlarged teeth, or that the importance of canine teeth in displays of male-to-male aggression waned with the development of weapons. *Ar. ramidus* negates such hypotheses because it demonstrates that small canines occurred in hominids long before any of the dental modifications of *Australopithecus* or the use of stone tools. The loss of large canine teeth in males must have occurred within the context of a generalized, nonspecialized diet. Comparisons of the *Ar. ramidus* dentition with those of all other higher primates indicate that the species retained virtually no anatomical correlates of male-to-male conflict. Consistent with a diminished role of such agonism, the body size of *Ar. ramidus* males was only slightly larger than that of females.

The discovery of *Ar. ramidus* also requires rejection of theories that



Breakthrough adaptations can transform life-history by deviating from typical reproductive strategy. Early hominids show feminized male canines [left] and primitive bipedality [right]. These suggest that females preferred nonaggressive males who gained reproductive success by obtaining copulation in exchange for valuable foods (vested provisioning). Success would depend on copulatory frequency with mates whose fertility remained cryptic (e.g., absence of cycling in mammary size). The result would be reduced agonism in unrelated females, and cooperative expansion of day ranges among equally cooperative males, eventually leading to exploitation of new habitats.

presume a chimpanzee- or gorilla-like ancestor to explain habitual upright walking. *Ar. ramidus* was fully capable of bipedality and had evolved a substantially modified pelvis and foot with which to walk upright. At the same time, it preserved the ability to maneuver in trees, because it maintained a grasping big toe and a powerful hip and thigh musculature. Because upright walking provided no energy advantage for *Ar. ramidus* (it lacked many of the adaptations evolved in later hominids such as *Australopithecus*), reproductive success must have been central to its evolution in early hominids.

Loss of the projecting canine raises other vexing questions because this tooth is so fundamental to reproductive success in higher primates. What could cause males to forfeit their ability to aggressively compete with other males? What changes paved the way for the later emergence of the energy-thirsty brain of *Homo*? Such questions

can no longer be addressed by simply comparing humans to extant apes, because no ape exhibits an even remotely similar evolutionary trajectory to that revealed by *Ardipithecus*.

When the likely adaptations of early hominids are viewed generally rather than with specific reference to living chimpanzees, answers to such questions arise naturally. Many odd hominid characteristics become transformed from peculiar to commonplace. Combining our knowledge of mammalian reproductive physiology and the hominid fossil record suggests that a major shift in life-history strategy transformed the social structure of early hominids. That shift probably reduced male-to-male conflict and combined three previously unseen behaviors associated with their ability to exploit both trees and the land surface: (i) regular food-carrying, (ii) pair-bonding, and (iii) reproductive cryptis (in which females did not advertise ovulation, unlike the case in chimpanzees). Together, these behaviors would have substantially intensified male parental investment—a breakthrough adaptation with anatomical, behavioral, and physiological consequences for early hominids and for all of their descendants, including ourselves.

See pages 62–63 for authors' affiliation.

When citing, please refer to the full paper, available at DOI 10.1126/science.1175834.

Ardipithecus ramidus and the Paleobiology of Early Hominids

Tim D. White,^{1*} Berhane Asfaw,² Yonas Beyene,³ Yohannes Haile-Selassie,⁴ C. Owen Lovejoy,⁵ Gen Suwa,⁶ Giday WoldeGabriel⁷

Hominid fossils predating the emergence of *Australopithecus* have been sparse and fragmentary. The evolution of our lineage after the last common ancestor we shared with chimpanzees has therefore remained unclear. *Ardipithecus ramidus*, recovered in ecologically and temporally resolved contexts in Ethiopia's Afar Rift, now illuminates earlier hominid paleobiology and aspects of extant African ape evolution. More than 110 specimens recovered from 4.4-million-year-old sediments include a partial skeleton with much of the skull, hands, feet, limbs, and pelvis. This hominid combined arboreal palmigrade clambering and careful climbing with a form of terrestrial bipedality more primitive than that of *Australopithecus*. *Ar. ramidus* had a reduced canine/premolar complex and a little-derived cranial morphology and consumed a predominantly C₃ plant-based diet (plants using the C₃ photosynthetic pathway). Its ecological habitat appears to have been largely woodland-focused. *Ar. ramidus* lacks any characters typical of suspension, vertical climbing, or knuckle-walking. *Ar. ramidus* indicates that despite the genetic similarities of living humans and chimpanzees, the ancestor we last shared probably differed substantially from any extant African ape. Hominids and extant African apes have each become highly specialized through very different evolutionary pathways. This evidence also illuminates the origins of orthograde, bipedality, ecology, diet, and social behavior in earliest Hominidae and helps to define the basal hominid adaptation, thereby accentuating the derived nature of *Australopithecus*.

In 1871, Charles Darwin concluded that Africa was humanity's most probable birth continent [(1), chapter 7]. Anticipating a skeptical reception of his placement of *Homo sapiens* as a terminal twig on the organic tree, Darwin lamented the mostly missing fossil record of early hominids (2). Following T. H. Huxley, who had hoped that "the fossilized bones of an Ape more anthropoid, or a Man more pithecoïd" might be found by "some unborn paleontologist" [(3), p. 50], Darwin observed, "Nor should it be forgotten that those regions which are the most likely to afford remains connecting man with some extinct ape-like creature, have not as yet been searched by geologists." He warned that without fossil evidence, it was "useless to speculate on this subject" [(1), p. 199].

Darwin and his contemporaries nonetheless sketched a scenario of how an apelike

ancestor might have evolved into humans. That scenario easily accommodated fossil evidence then restricted to European Neandertals and *Dryopithecus* (a Miocene fossil ape). Javanese *Homo erectus* was found in the 1890s, followed by African *Australopithecus* in the 1920s. By the 1960s, successive grades of human evolution were widely recognized. *Australopithecus* comprised several Plio-Pleistocene small-brained species with advanced bipedality. This grade (adaptive plateau) is now widely recognized as foundational to more derived *Homo*.

Molecular studies subsequently and independently confirmed Huxley's anatomically based phylogeny linking African apes and living humans (4). They also challenged age estimates of a human/chimpanzee divergence, once commonly viewed as exceeding 14 million years ago (Ma). The latter estimates were mostly based on erroneous interpretations of dentognathic remains of the Miocene fossil ape *Ramapithecus*, combined with the presumption that extant chimpanzees are adequate proxies for the last common ancestor we shared with them (the CLCA).

The phylogenetic separation of the lineages leading to chimpanzees and humans is now widely thought to have been far more recent. During the 1970s, discovery and definition of *Australopithecus afarensis* at Laetoli and Hadar extended knowledge of hominid biology deep into the Pliocene [to 3.7 Ma (5, 6)]. The slightly earlier (3.9 to 4.2 Ma) chronospecies *Au. anamensis* was subsequently recognized as another small-brained biped with notably large postcanine teeth and postcranial derivations shared with its apparent

daughter species (7, 8). Late Miocene hominid fossils have been recently recovered from Ethiopia, Kenya, and Chad. These have been placed in three genera [*Ardipithecus* (9–12), *Orrorin* (13), and *Sahelanthropus* (14)]. They may represent only one genus (12, 15), and they challenge both savanna- and chimpanzee-based models (16) of hominid origins.

Continuing to build on fossil-free expectations traceable to Darwinian roots, some hold that our last common ancestors with African apes were anatomically and behaviorally chimpanzee-like (17), that extant chimpanzees can be used as "time machines" (18), and/or that unique features of *Gorilla* are merely allometric modifications to accommodate its great body mass. Thus, early *Australopithecus* has routinely been interpreted as "transitional" and/or a "locomotor missing link" (19, 20) between extant humans and chimpanzees. Bipedality is widely suggested to have arisen as an opportunistic, or even necessary, response to a drier climate and the expansion of savannas. These views have been challenged on paleontological and theoretical grounds (9, 21). However, without additional fossil evidence, the evolutionary paths of the various great apes and humans have remained shrouded.

In related papers in this issue (22–27), we describe in detail newly discovered and/or analyzed specimens of *Ar. ramidus*, including two individuals with numerous postcranial elements. All are dated to 4.4 Ma and come from the Middle Awash area of the Ethiopian Afar rift. Local geology and many associated fossils are also described (28–30). These new data jointly establish *Ardipithecus* as a basal hominid adaptive plateau preceding the emergence of *Australopithecus* and its successor, *Homo*. Inferences based on *Ar. ramidus* also facilitate understanding its precursors (22, 23, 27, 31). Here, we provide an integrated view of these studies and summarize their implications.

The Middle Awash. The Middle Awash study area contains a combined thickness of >1 km of Neogene strata. To date, these deposits have yielded eight fossil hominid taxa spanning the Late Miocene to Pleistocene (>6.0 to <0.08 Ma) (32, 33). Hominids make up only 284 of the 18,327 total cataloged vertebrate specimens. Spatially and chronologically centered in this succession, the Central Awash Complex (CAC) (28, 34) rises above the Afar floor as a domelike structure comprising >300 m of radioisotopically and paleomagnetically calibrated, sporadically fossiliferous strata dating between 5.55 and 3.85 Ma. Centered in its stratigraphic column are two prominent and widespread volcanic marker horizons that encapsulate the Lower Aramis Member of the Sagantole Formation (Fig. 1). These, the Gåala ("camel" in Afar language) Vitric Tuff Complex (GATC) and the superimposed Daam Aatu ("baboon" in Afar language) Basaltic Tuff (DABT), have indistinguishable laser fusion ³⁹Ar/⁴⁰Ar dates of 4.4 Ma. Sandwiched between

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the two tuffs are fossiliferous sediments averaging ~3 m in thickness and cropping out discontinuously over an arc-shaped, natural erosional transect of >9 km (28). The rich fossil and geologic data from these units provide a detailed characterization of the Pliocene African landscape inhabited by *Ardipithecus*.

We first surveyed the CAC during 1981 in attempts to understand the distribution of fossils within the region. We launched a systematic program of geological, geochronological, and paleontological investigation in 1992. Initial visits to the CAC's northeastern flank documented abundant fossilized wood and seeds in the interval between the two tuffs. We collected and identified a highly fragmented sample of vertebrates, including abundant cercopithecoid monkeys and tragelaphine bovids. The first hominid fossils were found at Aramis vertebrate paleontology locality 1 (ARA-VP-1) on 17 December 1992. Two initial seasons of stratigraphic and geochronological studies yielded 649 cataloged vertebrates, including a minimum number of 17 hominid individuals represented mostly by teeth (10).

Because of its content, the Lower Aramis Member became the focus of our paleontological

efforts. Fourteen sublocalities within the original ARA-VP-1 locality were circumscribed and subjected to repeated collecting of all biological remains, based on multiple team crawls (35) across the eroding outcrops between 1995 and 2005. Analogous collections were made at adjacent localities (ARA-VP-6, -7, and -17), as well as at the eastern and western exposures of the *Ardipithecus*-bearing sedimentary units (KUS-VP-2 and SAG-VP-7) (KUS, Kuseralee Dora; SAG, Sagantole). The Lower Aramis Member vertebrate assemblage (table S1) now totals >6000 cataloged specimens, including 109 hominid specimens that represent a minimum of 36 individuals. An additional estimated 135,000 recovered fragments of bone and teeth from this stratigraphic interval are cataloged by locality and taxon as pooled "bulk" assemblages. Analogous samples were collected from the Lower Aramis Member on the eastern transect pole (SAG-VP-1, -3, and -6). Fossils from localities higher and lower in the local Middle Awash succession (7, 12, 32) and at nearby Gona (36) are reported elsewhere.

The ARA-VP-6/500 partial hominid skeleton.

Bones of medium and large mammals were usually ravaged by large carnivores, then embedded

in alluvial silty clay of the Lower Aramis Member. Once exposed by erosion, postdepositional destruction of the fossils by decalcification and fracture is typical. As a result, the larger vertebrate assemblage lacks the more complete cranial and postcranial elements typically recovered from other African hominid localities. The identification of larger mammals below the family level is therefore most often accomplished via teeth. The hominid subassemblage does not depart from this general preservational pattern (29).

There was consequently little initial hope that the stratigraphic interval between the two tuffs would yield crucially needed postcranial elements of *Ardipithecus*. The only relevant postcrania (arm elements) had come from slightly higher in the section in 1993 (10). However, on 5 November 1994, Y.H.S. collected two hominid metacarpal fragments (ARA-VP-6/500-001a and b) from the surface of an exposed silty clay ~3 m below the upper tuff (DABT), 54 m to the north of the point that had 10 months earlier yielded the *Ardipithecus* holotype dentition. Sieving produced additional hominid phalanges. The outcrop scrape exposed a hominid phalanx in situ, followed by a femur shaft and nearly complete tibia. Subsequent excavation during 1994

Fig. 1. Geography and stratigraphy of the Aramis region. Two dated volcanic horizons constrain the main *Ardipithecus*-bearing stratigraphic interval in the Aramis region. The top frame shows these tephra in situ near the eastern end of the 9-km outcrop. The dark stripe in the background is the riverine forest of the modern Awash River running from right to left, south to north, through the Middle Awash study area of the Afar Rift. The lower frames are contemporaneous helicopter views over ARA-VP-1 (Yonas Molar Site) to show the geographic position of the top photo and to depict the extensive outcrop of the upper tuff horizon (dotted lines show the DABT) across the local landscape. Vehicles are in the same position to provide orientation. Sediments outcropping immediately below this 4.4-million-year-old horizon yielded the floral, faunal, and isotopic contexts for *Ar. ramidus*. The frame to the left shows the slight eastward dip of the Sagantole Formation toward the modern Awash River. The contiguous frame to the right is a view up the modern upper Aramis catchment. The ARA-VP-6 locality where the partial *Ardipithecus* skeleton was excavated is near its top right corner (Fig. 2).



and the next field season (at a rate of ~ 20 vertical mm/day across ~ 3 m²) revealed >100 additional in situ hominid fragments, including sesamoids (Fig. 2 and table S2). Carnivore damage was absent.

The bony remains of this individual (*ARA-VP-6/500*) (Fig. 3) (37) are off-white in color and very poorly fossilized. Smaller elements (hand and foot bones and teeth) are mostly undistorted, but all larger limb bones are variably crushed. In the field, the fossils were so soft that

they would crumble when touched. They were rescued as follows: Exposure by dental pick, bamboo, and porcupine quill probe was followed by in situ consolidation. We dampened the encasing sediment to prevent desiccation and further disintegration of the fossils during excavation. Each of the subspecimens required multiple coats of consolidant, followed by extraction in plaster and aluminum foil jackets, then additional consolidant before transport to Addis Ababa.

Pieces were assigned number suffixes based on recovery order. Back-dirt was weathered in place and resieved. The 1995 field season yielded facial fragments and a few other elements in northern and eastern extensions of the initial excavation. Further excavation in 1996 exposed no additional remains. Each fragment's position, axial orientation, and dip were logged relative to a datum (strata here dip east at $\sim 4^\circ$ to 5°). A polygon representing the outer perimeter and vertical extent of the hominid fragment constellation (based on each bone's center point) was demarcated by a carapace of limestone blocks cemented with concrete after excavation, then further protected by a superimposed pile of boulders, per local Afar custom.

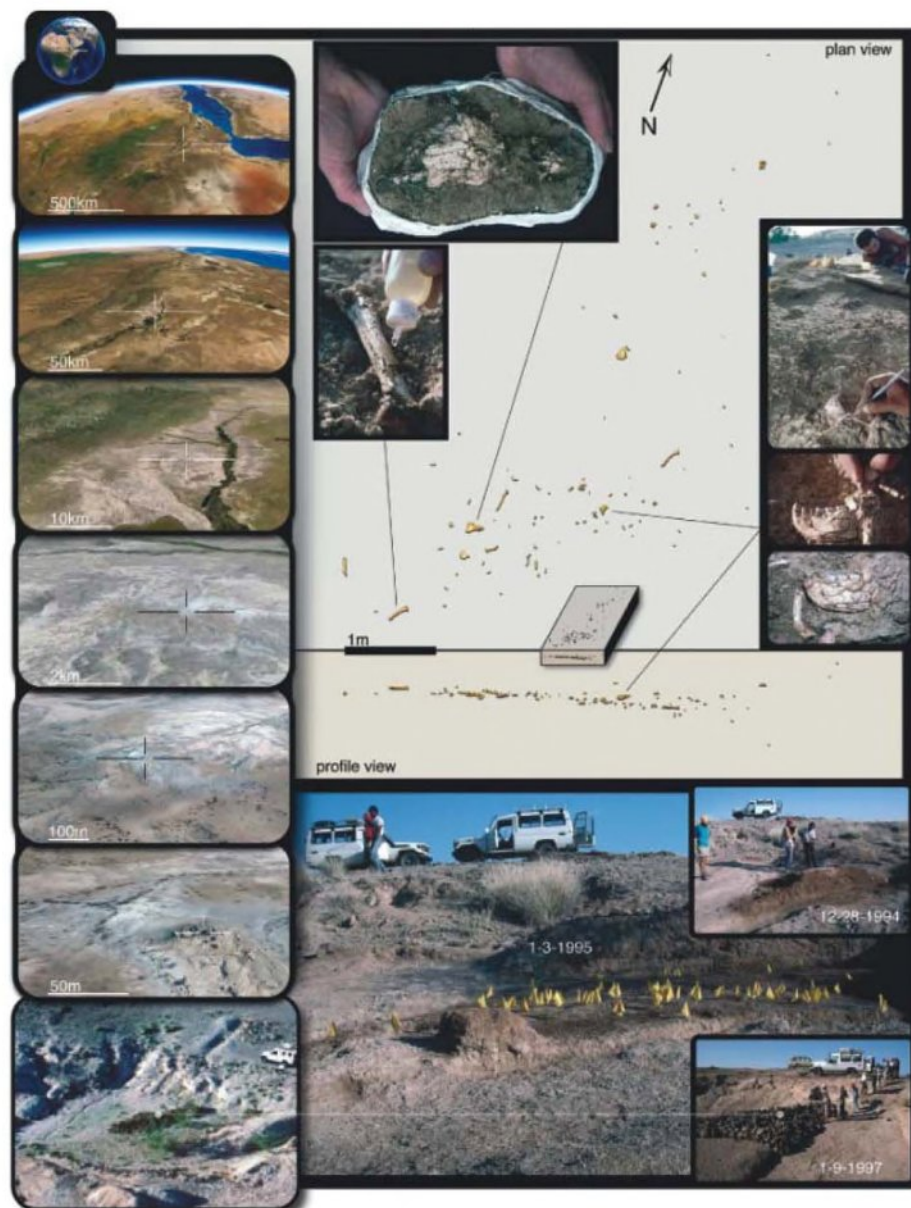


Fig. 2. The *ARA-VP-6/500* skeletal excavation. Successive zooms on the *ARA-VP-6/500* partial skeleton discovery are shown. Insets show the application of consolidant to the tibia shaft and removal of the os coxae in a plaster jacket in 1994–1995. No skeletal parts were found articulated (the mandible excavation succession shows the close proximity of a proximal hand phalanx and trapezium). Only in situ specimens are shown on the plan and profile views. Note the tight vertical and wider horizontal distributions of the remains. Local strata dip $\sim 5^\circ$ to the east. The lower inside corner of each yellow pin flag marks the center point for each in situ specimen from the 1994–1995 excavation. The 1995–1996 excavation recovered additional, primarily craniodental remains between these flags and the vehicle. The boulder pile emplaced at the end of the 1996–1997 excavation marks the discovery site today.



Fig. 3. The *ARA-VP-6/500* skeleton. This is a composite photograph to show the approximate placement of elements recovered. Some pieces found separately in the excavation are rejoined here. Intermediate and terminal phalanges are only provisionally allocated to position and side.

The skeleton was scattered in typical Lower Aramis Member sediment (Fig. 2): fine-grained, massive, unlickensided, reddish-brown alluvial silty clay containing abundant decalcified root casts, fossil wood, and seeds. A 5- to 15-cm lens of poorly sorted sand and gravel lies immediately below the silty clay, and the spread of cranial parts to the north suggests that the bones of the carcass came to rest in a shallow swale on the floodplain.

There is no evidence of weathering or mammalian chewing on *ARA-VP-6/500*. Bony elements were completely disarticulated and lacked anatomical association. Many larger elements showed pre-fossilization fragmentation, orientation, and scatter suggestive of trampling. The skull was particularly affected, and the facial elements and teeth were widely scattered across the excavated area. Bioturbation tilted some phalanges and metacarpals at high dip angles (Fig. 2). A few postcrania of a large *Aquila* (eagle) and other birds were recovered during excavation, as were a few micromammals. No large-mammal remains (except isolated cercopithecoid teeth and shaft splinters from a medium-to-large mammal limb bone) were associated. The cause of death is indeterminate. The specimen is judged to be female. The only pathology is a partially healed osteolytic lesion suggestive of local infection of the left proximal ray 5 pedal phalanx (*ARA-VP-6/500-044*).

Laboratory exposure and consolidation of the soft, crushed fossils were accomplished under binocular microscope. Acetone was applied with brushes and hypodermic needles to resoften and remove small patches of consolidant-hardened encasing matrix. Microsurgery at the interface between softened matrix and bone proceeded millimeter by submillimeter, rehardening each cleaned surface with consolidant after exposure. This process took several years. The freed specimens remain fragile and soft, but radiographic accessibility is excellent. Most restoration and correction for distortion were accomplished with plaster replicas or micro-computed tomography digital data to preserve the original fossils in their discovery state.

Environmental context. The Lower Aramis Member lacks any evidence of the hydraulic mixing that afflicts many other hominid-bearing assemblages. The unwarranted inference that early hominids occupied “mosaic habitats” (38) is often based on such mixed assemblages, so the resolution and fidelity of the Aramis environmental data sets are valuable. We estimate that the interval of time represented by the strata between the two tuffs at Aramis is $<10^5$ years, and perhaps just a few hundred or thousand years (28, 39). The lithology, thickness, taphonomic evidence, and similar age of the constraining marker horizons imply that geologically, the evidence can be viewed as “habitat time-averaged” (40). Indeed, we do not see notably different environmental indicators in the fossils or geologic or chemical data sampled vertically

throughout the interval. The wealth of data allows a high-fidelity representation [sensu (41)] of the ecological community and environment inhabited by *Ar. ramidus* 4.4 Ma.

A variety of data indicate that the wooded biotope varied laterally across the Pliocene landscape (28–30). The hominid-bearing localities (centered on the *ARA-VP-1* sublocalities) are rich in fossilized wood fragments, seeds, and animal fossils. Here, isotopic paleosol compositions indicate mostly wooded conditions (28). There was obviously more water at Aramis then (4.4 Ma)—supporting a much richer flora and fauna—than there is today. The higher water budget is possibly due to higher elevation during deposition (42) or to paleoclimatic factors such as a more continuous Pliocene El Niño effect (43). An abrupt transition occurs southeast of the *SAG-VP-7* locality, where sedimentary, faunal, taphonomic, and isotopic data imply a more open rift-axial setting depauperate in faunal remains and lacking in primates, micromammals, and macrobotanical remains (29, 30).

Along the northern slope of the CAC, all localities of the Lower Aramis Member yielded tragelaphine bovids, monkeys, and other data indicative of more wooded conditions. Carbon isotopes from the teeth of five *Ardipithecus* individuals found here imply that they fed largely on C_3 plants in woodlands and/or among the small patches of forests in the vicinity. We interpret the combined contextual data to indicate that *Ar. ramidus* preferred a woodland-to-forest habitat (29, 30) rather than open grasslands. This finding is inconsistent with hypotheses positing hominid origins via climate-driven savanna expansion.

Variation and classification. Initial (1994) description of the limited hominid sample from Aramis placed these remains in a newly discovered *Australopithecus* species interpreted as the most primitive then known (10). Subsequent recovery of the *ARA-VP-6/500* skeleton showed that, relative to body size, its dentition was small, unlike *Australopithecus*. Strict cladistic practice required a new genus name for this sister taxon of *Australopithecus*, so the material was renamed as the new genus *Ardipithecus* in 1995, with the lack of megadonty added to the species diagnosis even as the partial skeleton’s excavation was still under way (44). Subsequent discovery of the earlier probable chronospecies *Ar. kadabba* in 1997 (11, 12) was followed by recovery of *Orrorin* in 2000 (13) and *Sahelanthropus* in 2001 (14). These Late Miocene fossils provide additional outgroup material useful in assessing the phylogenetic position of *Ar. ramidus*.

Only two adjacent Ethiopian study areas (the Middle Awash and Gona) have yielded confirmed remains of *Ar. ramidus* to date (7, 36). Neither has produced any evidence to reject a single species lineage as the source of the combined hominid sample from these Pliocene sites. We thus interpret the Lower Aramis Member hominid assemblage as a single taxon (22). Pene-

contemporary (~4.3 to 4.7 Ma) hominid remains from elsewhere are sparse (45, 46), and these are broadly compatible with the now expanded range of variation in *Ar. ramidus* (22, 23). Thus, although continental sampling is still obviously inadequate, describing hominid species diversity in this time frame (47) as “very bushy” seems unwarranted (48).

The amount of variation within the known Afar *Ar. ramidus* sample appears to be lower than typical for species of *Australopithecus*. This is probably due to a lesser degree of sexual dimorphism in *Ardipithecus*, combined with the narrow time window represented by the interval between the two Aramis tuffs. Skeletal dimorphism is notably difficult to assess, except in rare instances of geologically isochronous samples of a species lineage (e.g., A.L. 333 “first family”) (49). For *Ar. ramidus*, the *ARA-VP-6/500* skeleton (Figs. 3 and 4) provides a rare opportunity for guiding a probabilistic approach to sex attribution of conspecific fossils, relying on canines (22) and postcranially based estimates of body size (27). The implication is that there was broad overlap in body size between males and females of *Ar. ramidus*.

Cranial and dental anatomy. The *Ar. ramidus* skull (23) is very similar to the larger, more robust *Sahelanthropus* cranium (*TM 266-01-60-1*) from Chad, also interpreted as an early hominid (14, 50). Some of the differences are probably partly sex-related. *Ar. ramidus* shares with *Sahelanthropus* a small cranial capacity (300 to 350 cc) and considerable midfacial projection but a maxillo-premaxillary complex that is less prognathic than that of modern African apes [not necessarily a derived trait shared with *Homo*, in contrast with (51)]. The *Ardipithecus* and *Sahelanthropus* crania each lack a distinct post-toral sulcus, and both exhibit an anteriorly positioned posterior cranial base.

Most aspects of the craniofacial structure of *Sahelanthropus/Ardipithecus* are probably close to the African ape and hominid ancestral state. Gorilla and chimpanzee cranial morphologies, as well as their specialized dentitions, are clearly divergently derived (22). In *Gorilla*, enhanced facial size and prognathism occur in relation to larger general size and an increasing adaptation to herbivory and folivory. In *Pan* (also with enhanced prognathism), derived cranial form (including anterior basicranial lengthening) probably occurred as a part of enhanced terrestriality accompanied by elevated agonistic behavior and its anatomical correlates, such as tusklike canines (22, 23). The bonobo cranial base and *Ardipithecus* craniofacial structure may be less derived, but even the bonobo seems to be derived in its relatively small face and global dental reduction (22). This was probably at least in part due to decreased intraspecific aggression in the bonobo lineage after separation from the common chimpanzee lineage.

The superoinferiorly short but intermediately prognathic *Ar. ramidus* face lacks the

broadening and anterior migration of the zygomatic area seen to varying degrees in species of *Australopithecus*. The primitive craniofacial pattern shared between *Sahelanthropus* and *Ardipithecus* suggests that the genus *Australopithecus* would later evolve a craniofacial struc-

ture capable of increased postcanine mastication consequent to an ecological breakout from wooded habitats, expanding its foraging into more open environments (7, 10).

The *Ardipithecus* dentition suggests omnivory (22). It exhibits none of the specializations

seen among modern apes; neither the large incisors of *Pongo* or *Pan* nor the specialized molar morphology of *Pongo*, *Pan*, or *Gorilla*. Postcanine size relative to body size was slightly larger than in *Pan* but smaller than in *Gorilla*, *Pongo*, or (especially) *Au. afarensis*. *Ar. ramidus* molars overlap considerably with *Pan* in some measures of enamel thickness but differ in overall thickness and structure. Chimpanzee molars have a broad occlusal basin with locally thin enamel not seen in *Ardipithecus*. *Pan* molar morphology is probably an adaptation to crushing relatively soft and nonabrasive food items such as ripe fruits, while retaining some shearing capacities. The *Ardipithecus* dentition shows no strong signals of ripe-fruit frugivory, folivory-herbivory, or feeding on hard objects. Its macroscopic and microscopic wear patterns, as well as the low bunodont cusps with intermediate enamel thickness (22), suggest that its diet was not particularly abrasive but may have included some hard foods. It is consistent with a partially terrestrial, partially arboreal pattern of feeding in a predominantly wooded habitat.

Carbon isotopic evidence from the teeth of five *Ar. ramidus* individuals suggests that *Ardipithecus* and *Australopithecus* were distinct in dietary intake (30). "Robust" and "nonrobust" *Australopithecus* have enamel isotope values indicating a diet of more than 30% C_4 plants, with variation ranging up to ~80% C_4 . In contrast, the known *Ar. ramidus* individuals vary only between ~10 and 25% C_4 , and thus also differ from *Pan troglodytes*, which prefers ripe fruit and is considered closer to a pure C_3 feeder (30). Thus, *Ardipithecus* appears to have exploited a wider range of woodland resources than do chimpanzees, but without relying on the open biotope foods consumed by later *Australopithecus*.

Evolution of the canine/lower third premolar complex (C/P_3) potentially illuminates social and reproductive behavior. The *Ar. ramidus* canine sample totals 21 Aramis individuals. Some are small fragments, but all show informative morphology and/or wear. All specimens are either morphologically similar to those from female apes or are further derived toward the later hominid condition (22). Morphological and metric variation in the sample is small. Functionally important sex-related size dimorphism is not apparent. There is no evidence of functional honing (planar facets on the mesiobuccal P_3 or sharpened edges on the distolabial upper canine margin). The largest, presumably male, specimens are as morphologically derived as the smallest, showing that dimorphic canine morphology was virtually absent in these hominids by 4.4 Ma. Furthermore, a juvenile probable male lacks the delayed canine eruption seen in chimpanzees, approximating the *Au. anamensis* and *Au. afarensis* conditions and indicating that the canine was not an important component of adult sociobehavioral relationships.

The differential status of upper versus lower canine morphology is informative. In *Ar. ramidus*,

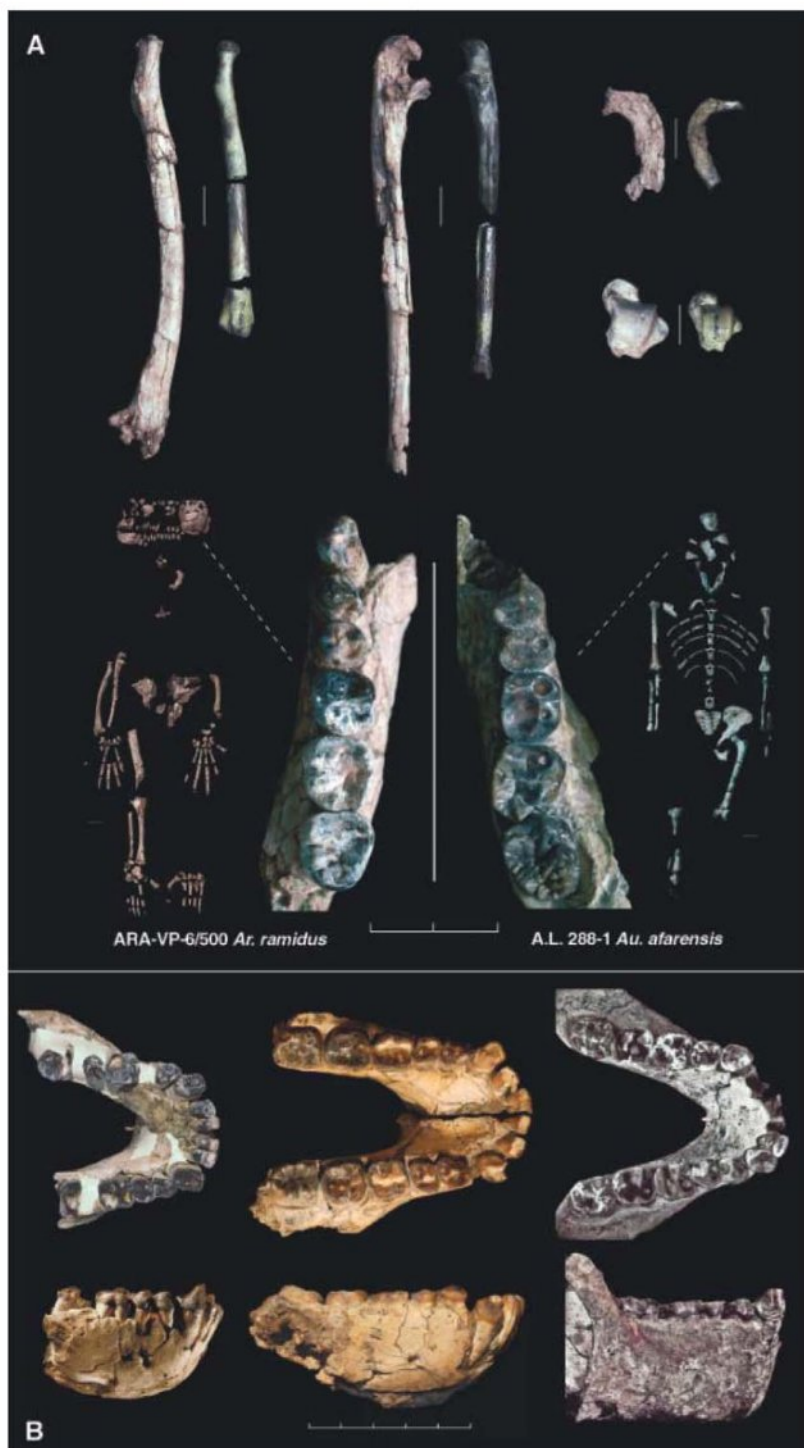


Fig. 4. Comparisons of *Ardipithecus* (left) and early *Australopithecus* (right). (A) Ulnar, radial, first rib, and talar comparisons of the *Ar. ramidus* ARA-VP-6/500 and *Au. afarensis* A.L. 288-1 ("Lucy") skeletal individuals illustrate larger postcranial dimensions for the *Ardipithecus* individual relative to dental size. Comparison of the postcanine dentitions reveals the megadontia of the *Australopithecus* individual. (B) Occlusal and lateral views of three time-successive mandibles dated to 4.4, 4.12, and 3.4 Ma, respectively, from left to right: ARA-VP-1/401 *Ar. ramidus*; KNM-KP 29281 *Au. anamensis* holotype (mirrored); MAK-VP-1/12 *Au. afarensis* (mirrored).

the lower canines retain modally more apelike morphology than do the uppers, and, in contradistinction to other anthropoids, the height of the maxillary canine crown is lower than that of the mandibular (22). This relationship is opposite that seen in great apes and cercopithecids, whose upper canine dominance is exaggerated, particularly in males of dimorphic species. In these primates, upper canine projection and prominence function in both weaponry and display. The *Ar. ramidus* canines are metrically and morphologically derived in the direction of later hominids, and we hypothesize that reduction and alteration of upper canine size and shape in this and earlier hominid species are related to changes in social behaviors (22, 31).

The canines of *Sahelanthropus*, *Orrorin*, and *Ar. kadabba* are broadly equivalent to those of *Ar. ramidus* in size and function. However, the upper canines of Late Miocene hominids exhibit a subtle but distinctly more primitive morphology than their *Ar. ramidus* homologs, potentially including occasional residual (female ape-like) honing as part of their variation (12, 15). This suggests that upper canine prominence was reduced through the Late Miocene and Early Pliocene. In contrast, the C/P₃ complex of the last common ancestor of hominids and chimpanzees probably had a moderate level of canine dimorphism combined with functional honing. This was subsequently generally retained in *P. paniscus* and enhanced in *P. troglodytes*.

Body size and dimorphism. The partial skeleton *ARA-VP-6/500* is identified as female based on probability assessments of canine size (its canines are among the smallest of those of 21 available individuals) (22). This interpretation is corroborated by its small endo- and exocranial size, as well as its superoinferiorly thin supra-orbital torus (23). Bipedal standing body height for the *ARA-VP-6/500* individual is estimated at approximately 120 cm, and body mass at ~50 kg (27). Although actual body mass may vary considerably in relation to skeletal size, this is a large female body mass.

Of the *Ar. ramidus* postcranial elements, the humerus represents the largest minimum number of individuals (seven). *ARA-VP-6/500* does not preserve a humerus, but detailed comparisons suggest that its forelimb was ~2 to 8% larger in linear dimensions than the partial forelimb skeleton *ARA-VP-7/2* (24, 27), which does include a humerus. This would make *ARA-VP-6/500* either the second- or third-largest of eight individuals within the Aramis humeral sample. The combined evidence suggests that *Ardipithecus* skeletal body size was nearly monomorphic, and less dimorphic than *Australopithecus*, as estimated from template bootstrapping (49). Most likely, *Ardipithecus* exhibited minimal skeletal body size dimorphism, similar to *Pan*, consistent with a male-bonded social system, most likely a primitive retention from the CLCA condition (31). With its subsequent commitment to terrestrial bipedality, *Australopithecus* probably enhanced

female cooperation and group cohesion, thus potentially reducing female body size, whereas male size increased in response to predation pressure, probably elevated by expanding niche breadth.

Postcranial biology and locomotion. Regardless of whether the Afar *Ar. ramidus* population represents a hominid relict or a lineal ancestor, this taxon's biology resolves fundamental evolutionary questions persisting since Darwin. Its substantially primitive postcranial anatomy appears to signal a grade-based difference from later *Australopithecus*. The challenge of understanding its evolutionary and functional implications required a nontraditional approach. Without testable hypotheses of underlying gene-based developmental mechanisms, many paleo-anthropological analyses have been adaptationist (52) and/or purely numerically discriminatory. Therefore, wherever possible, in the accompanying postcranial papers (24–27) we restrict hypotheses to those that can be formulated consistent with putative selection acting on cascades of modular-based positional information, especially when these can be potentially grounded in known anabolic mechanisms. This approach is summarized elsewhere (53, 54) and in supporting online material text S1.

The upper pelvis of *Ar. ramidus* presents a contrast to its primitive hand, foot, and limbs. The ilia are abbreviated superoinferiorly and sagittally oriented but broad mediolaterally, so much so that the anterior inferior iliac spine has become a separate growth site, as in all later hominids. The pubic symphyseal face is quite short. A slight sciatic notch is present, although ischial structure was similar to that of extant African apes. This suggests that pattern-formation shifts for bipedality were only partly realized in *Ar. ramidus*. These changes may have culminated a long period of facultative bipedality hinted at by isolated postcranial elements from the probable chronospecies *Ar. kadabba* (12) and other Late Miocene forms (13, 14).

Paramount among the retained primitive characters of the *Ar. ramidus* hindlimb is a fully abductable first ray (hallux, or great toe), but in combination with elements of a robust plantar substructure that stabilized the foot during heel- and toe-off. Although it was still a highly effective grasping organ, the foot of *Ar. ramidus* also maintained propulsive capacity long since abandoned by extant great apes (in which greater opposition between the hallux and lateral rays evolved, i.e., a more handlike conformation than in *Ar. ramidus*) (26).

Other defining and notably primitive characters include a moderately elongate mid-tarsus, a robust lateral peroneal complex in which muscles of the lateral compartment performed substantial plantarflexion, and a primitive (flexion-resistant) geometric configuration of the lateral metatarsal bases. Thus, the *Ar. ramidus* foot is an amalgam of retained primitive characters as well as traits specialized for habitual

bipedality, such as the expanded second metatarsal base that anchored plantarflexion during heel- and toe-off. Many of the foot's primary adaptations to fulcrumation are probable retentions from the gorilla/chimpanzee/human last common ancestor (GLCA), but these have been eliminated in apes, presumably for vertical climbing.

The *ARA-VP-6/500* radius/tibia ratio is 0.95, as in generalized above-branch quadrupeds such as macaques and *Proconsul* (an Early Miocene ape) (27). Its intermembral index (the ratio of forelimb length to hindlimb length) is also similar to those of above-branch quadrupeds. These facts suggest that African apes experienced both forelimb elongation and hindlimb reduction, whereas hominid proportions remained largely unchanged until the dramatic forearm shortening and hindlimb elongation of Plio-Pleistocene *Homo*.

These primitive proportions are consistent with virtually all other aspects of the *Ar. ramidus* skeleton. The inferred locomotor pattern combined both terrestrial bipedality and arboreal clambering in which much weight was supported on the palms. The hand phalanges are elongate relative to those of *Proconsul*, but metacarpals (Mc) 2 to 5 remained primitively short and lacked any corporal modeling or adaptations typical of knuckle-walking (24). Moreover, the virtually complete wrist of *ARA-VP-6/500* (lacking only the pisiform) exhibits striking adaptations for midcarpal dorsiflexion (backward deflection of the dorsum of the hand), consistent with a highly advanced form of arboreal palmigrady. In addition, substantial metacarpal-phalangeal dorsiflexion is indicated both by moderate dorsal notching of the Mc2 to -5 heads and by marked palmar displacement of the capitate head. Together these must have permitted dorsiflexion of the wrist and hand to a degree unparalleled in great apes.

The *Ar. ramidus* elbow joint provided full extension but lacks any characters diagnostic of habitual suspension. Ulnar withdrawal was complete and the thumb moderately robust, with indications of a distinct and fully functional flexor pollicis longus tendon. The hamate's hamulus permitted substantial metacarpal motion for opposition against the first ray. The central joint complex (Mc2/Mc3/capitate/trapezoid) exhibits none of the complex angular relationships and marked syndesmotomic reinforcement seen in extant apes. Together, these retained primitive characters, unlike their homologs in highly derived African apes, imply that the dominant locomotor pattern of the GLCA was arboreal palmigrady rather than vertical climbing and/or suspension (orthogrady). Another strong inference is that hominids have never knuckle-walked (26).

The extraordinary forelimb of *Ar. ramidus*, in combination with its limb proportions and likely primitive early hominid lumbar column (55), casts new light on the evolution of the lower spine. The traditional interpretation has

been that the lumbar transverse processes became dorsally relocated as the lumbar column reduced in length. The data from *Ar. ramidus* imply that ulnar withdrawal was not a suspensory adaptation but was instead an enhancement of distal forelimb maneuverability that accompanied profound changes in the shoulder. Spinal column invagination appears to have been an integral part of thoracic restructuring to increase shoulder joint laterality, thereby enhancing forelimb mobility for advanced arboreal quadrupedalism, especially careful climbing and bridging. A still primitive deltoid complex in both *Ar. ramidus* and Asian ancestral apes (e.g., *Sivapithecus*) now becomes more understandable. A predominantly Sharpey's fiber deltoid insertion can be viewed as a retention in above-branch quadrupeds that only later became modified for suspension (separately) in extant African and Asian apes.

The adoption of bipedality and its temporal association with progressive canine reduction and loss of functional honing now constitute the principal defining characters of Hominidae. The orthograde positional behaviors of hominids and apes were thus acquired in parallel, generated by early bipedal progression in the former and suspension and vertical climbing in the latter. Overall, *Ar. ramidus* demonstrates that the last common ancestors of humans and African apes were morphologically far more primitive than anticipated, exhibiting numerous characters reminiscent of Middle and Early Miocene hominoids. This reinforces what Huxley appreciated in 1860: "the stock whence two or more species have sprung, need in no respect be intermediate between those species" [(56), p. 568].

Ardipithecus and the great apes. *Ar. ramidus* illuminates several collateral aspects of hominoid evolution. Despite the demise of *Ramapithecus* as a putative hominid ancestor, at least one Eurasian Miocene ape, *Ouranopithecus*, has been suggested as being phyletically related to later African hominids (57), whereas another, *Dryopithecus*, is often considered an alternative sister taxon of the hominid and African ape clade (58). *Ardipithecus* effectively falsifies both hypotheses. *Ar. ramidus* lacks the derived characters of *Ouranopithecus* associated with postcanine enlargement and relative canine reduction while still providing a primitive morphological substrate for the emergence of *Australopithecus*. The new perspective that *Ar. ramidus* offers on hominoid postcranial evolution strongly suggests that *Dryopithecus* acquired forelimb adaptations to suspensory behaviors independently from African apes. *Ar. ramidus* suggests that these Eurasian forms were too derived to have been specially related to either the hominid or extant African ape clades. Moreover, the remarkably primitive postcranium of potential *Pongo* ancestors (e.g., *Sivapithecus*), coupled with what is now evidently widespread homoplasy in extant hominoids, suggests that the *Pongo* clade was established even before the first dispersal events of large-

bodied apes from Africa into Eurasia, shortly after docking of the Afro-Arabian and Eurasian plates at ~18 Ma (59).

An additional implication of *Ar. ramidus* stems from its demonstration that remarkable functional and structural similarities in the postcrania of *Pongo* and the African apes have evolved in parallel, as have those of *Pan* and *Gorilla* (27). Until now, a myriad of characters shared among the extant African apes were presumed to have been present also in ancestral hominids (because they were presumed to have been the ancestral state) (60). However, it now appears that many of these putative shared primitive characteristics have evolved independently. This highlights the alacrity with which similar anatomical structures can emerge, most likely by analogous selection operating on homologous genomes. The same genetic pathways can be repeatedly and independently coopted, resulting in convergent adaptations (61). Recent work on gene expression demonstrates that there are also multiple pathways that can produce similar but independently derived anatomical structures (62).

Work on deep homology shows that parallel evolution "must be considered a fact of life in the phylogenetic history of animals" [(63), p. 822]. This is also seen in more terminal branches; for example, during the past two million years of stickleback fish evolution (64). Such evolvability and parallelism are even suggested for the catarrhine dentition (65). *Ar. ramidus* reveals an excellent example of this phenomenon within the African ape-hominid clade by demonstrating the striking reoccurrence of syndesmotric fixation of the central joint complexes in hominoid wrists adapted to suspensory locomotion (including not only those of *Pan* and *Gorilla* but also those of *Pongo* and, partially, *Dryopithecus*). Such observations on very different evolutionary scales all caution against indiscriminant reliance on raw character states to assess phylogeny. A consideration of wider patterns of manifestations of such adaptive evolution, not only in character constellations but also in their evolutionary context, may be needed to tease apart homology and homoplasy. A far more complete fossil record will be needed to accomplish such a goal.

Such considerations also bear on current estimates of the antiquity of the divergence between the human and chimpanzee clades. Many such estimates, suggesting striking recency, have become widely accepted because of the presumed homology of human and African ape

morphologies (60). This obtains despite the recognition that broad assumptions about both the regularity of molecular change and the reliability of calibration dates required to establish such rates have strong limitations (66, 67). The homoplasy now demonstrated for hominoids by *Ar. ramidus* provides fair warning with respect to such chronologies, especially those currently used to calibrate other divergence events, including the split times of New and Old World monkeys, hylobatids, and the orangutan. The sparseness of the primate fossil record affecting these estimates is now compounded by the dangers posed by convergences perceived as homologies. Such difficulties are further exacerbated by newly recognized complexities in estimating quantitative degrees of genetic separation (66–68). In effect, there is now no a priori reason to presume that human-chimpanzee split times are especially recent, and the fossil evidence is now fully compatible with older chimpanzee-human divergence dates [7 to 10 Ma (12, 69)] than those currently in vogue (70).

Hominid phylogenetics. The expanded *Ar. ramidus* sample allows more detailed consideration of early hominid phylogenetics. The placement of *Ardipithecus* relative to later hominids can be approached by using modern and Miocene apes as the outgroup. An earlier cladistic study of this kind concluded that *Ar. ramidus* was the sister taxon of all later hominids (71). A more recent assessment of *Ar. ramidus* dental characters came to the same conclusion (7). In these analyses, a suite of derived features and character complexes exclusively aligning *Ar. ramidus* with *Australopithecus* was identified, but these were based on comparatively limited anatomical elements. The *Ar. ramidus* characters reported here, combined with those from Gona (36), allow a more complete analysis that clarifies the relationships among early hominid taxa.

Parsimony-based cladistic analyses are useful in deciphering relationships within the hominid family tree, despite their shortcomings (72, 73). The distribution of characters identified in Table 1 clearly shows that *Ar. ramidus* is derived relative to all known Late Miocene fossils attributed to the hominid clade. The earlier and more primitive probable chronospecies *Ar. kadabba* is found in 5.5- to 5.7-million-year-old deposits a mere 22 km west of Aramis, consistent with local (and perhaps regional) phyletic evolution. Its limited known elements are similar to those of other Late Miocene hominids in Kenya and Chad (12–14).

Table 1. (See pages 82 and 83.) The assembly of shared derived characters among early hominid taxa. Late Miocene and early Pliocene fossils now allow the strong inference of some character states (primitive, in blue) in the last common ancestor shared by chimpanzees and humans. Many other characters (not shown here) of extant apes were once considered primitive but are now shown to be derived and specific to those lineages. Late Miocene fossils from Ethiopia, Kenya, and Chad share some derived characters (in yellow) with all later hominids. The *Ar. ramidus* sample reported here shows a mixture of primitive and derived characters consistent with its phylogenetic and chronological placement. Phylogenetic implications are in Fig. 5. (An Excel version of this table is available in the supporting online material.)

Table 1. The assembly of shared derived characters among early hominid taxa.

Craniomandibular characters	Chimp/human LCA (INFERRED)	<i>Ar. kadabba/Sa. tchadensis/O. tugenensis</i>	<i>Ar. ramidus</i>	<i>Au. anamensis</i>	<i>Au. afarensis</i>
TMJ articular eminence	flat	flat	flat	TMJ with defined eminence	TMJ with defined eminence
Mandible corpus breadth	indeterminate	mandibular corpus broad	mandibular corpus broad	mandibular corpus broad	mandibular corpus broad
Mental foramen	indeterminate	circum mid-corpus ht weak	circum mid-corpus ht weak	circum mid-corpus ht intermediate	secondarily lowered lateral prominence developed
Mandibular lateral prominence	weak	root posterior, sulcus narrow	root posterior, sulcus narrow	intermediate	ramus root anterior and wide extramolar sulcus
Ramus root/ extramolar sulcus	root posterior, sulcus narrow	strong anterior	strong anterior	strong	bulbous (Laet.) to vertical (AL, MAK) anterior
Symphyseal inclination	slightly posterior	advanced?	advanced	indeterminate	advanced
Basion position	moderate midsagittal flexion, orbital kyphosis minimal	not extreme	not extreme	indeterminate	midfacial breadth greater
Cranial base flexion	moderate midsagittal flexion, orbital kyphosis minimal	zygomatotic root c. M1	zygomatotic root c. M1	zygomatotic root more anterior	zygomatotic root more anterior
Midfacial breadth	not extreme	indeterminate	present	absent	absent
Zygomatotic root	zygomatotic root c. M1	indeterminate	present	absent	absent
Incisor/lower canine step	present	indeterminate	present	absent	absent
Dental characters	Chimp/human LCA (INFERRED)	<i>Ar. kadabba/Sa. tchadensis/O. tugenensis</i>	<i>Ar. ramidus</i>	<i>Au. anamensis</i>	<i>Au. afarensis</i>
Sectorial C/P3 shearing	present, strong in males	sometimes present? in reduced expression?	absent	absent	absent
Canine size dimorphism	dimorphic	reduced C size dimorphism	further reduction?	further reduction	further reduction
Female relative canine size	moderate	moderate	moderate	slightly smaller	slightly smaller
Upper canine shape feminization	males unfeminized, higher crowned, modally lower shoulder	male C feminized in shape	male C feminized in shape	male C feminized in shape	male C feminized in shape
Shoulder height	females mostly mid to low	mostly mid to low?	mid to high	mid to high	sometimes extremely high
Shoulder flare	weak	weak	distinct flare	distinct flare	distinct flare
Lingual marginal ridge	weak	intermediate?	fold-like	fold-like	fold-like
Main mesial lingual ridge	strong (secondarily weak in Pan)	strong	basally broad	less prominent	more spatulate
Crown height	males tall, females moderate	indeterminate	UC height differentially reduced	reduced	reduced
Lower canine shape feminization	males higher crowned, modally low mesial shoulder, weak/no distal tubercle	feminized	feminized	feminized	feminized
Mesial shoulder height	females vary from low to high	varies from low to high	intermediate?	intermediate?	LC with high mesial shoulder
Lingual marginal ridge	weak or none	intermediate?	fold-like	fold-like	fold-like
Distal crest	usually weak or none	weak	weak	intermediate	distinct
Distal tubercle	weak	developed	developed	variable	distal tubercle less distinct merges with distal crest
Canine enamel thickness	thin	thin	thin	intermediate	thicker
Lower third premolar wear	hones UC	rarely hones, distal UC wear steep	No hone, distal UC steep	horizontal wear more dominant?	horizontal wear more dominant
Basal crown size/shape	obliquely elongate	intermediate?	elongation weaker, relatively smaller size	basally expanded and large	tends to be BL broader
Height	tall, with MB cervical extension	intermediate?	MB cervical extension weaker	low, squat, weak extension	weaker extension
Metaconid	absent or rudimentary	rudimentary	rudimentary	rudimentary	variably developed
Transverse crest	tall, near-transverse to posteriorly directed	near-transverse	near-transverse	near-transverse	more clearly transverse
Mesial marginal ridge	weak or none	intermediate?	distinct	distinct	tends to form developed anterior fovea
Upper third premolar	not developed, steep anterior face	weak delineation	better defined	better defined	tendency for more horizontal fovea
Anterior fovea	weak to moderate	weak to moderate	weak to moderate	weak to moderate	symmetry more frequent
Asymmetry	weak to moderate	weak to moderate	weak to moderate	weak to moderate	symmetry more frequent

Key: LCA → Primitive condition → Intermediate derived condition → Derived condition → Hominid clade → table continued

Table 1. The assembly of shared derived characters among early hominid taxa—continued.

Dental characters (continued)	Chimp/human LCA (INFERRED)	<i>Ar. kadabba/Sa. tchadensis/O. tugenensis</i>	<i>Ar. ramidus</i>	<i>Au. ananensis</i>	<i>Au. afarensis</i>
Lower deciduous molar					
<i>crown shape</i>	buccolingually narrow	indeterminate	buccolingually narrow	intermediate	broad, with developed anterior fovea
<i>protoconid dominance</i>	strong	indeterminate	strong	intermediate	larger metaconid
<i>talonid</i>	little developed	indeterminate	little developed	intermediate	posterior cusps well defined
Molars					
<i>lower molar shape</i>	indeterminate	relatively broader	relatively broader	relatively broader	tends to be very broad
<i>molar row length</i>	moderate	moderate	moderate	size increase	further increase
<i>lower M3 development</i>	variable, usually weak distal crown	variable, usually weak distal crown	variable, usually weak distal crown	large M3 with better developed distal crown	further LM3 complexity
<i>occlusal foveae</i>	moderately broad	moderately broad	moderately broad	narrower (increased basal flare)	narrower (increased basal flare)
<i>crown height</i>	low	low	low	intermediate?	taller M1 crown height
Molar enamel thickness	intermediate, variable	intermediate, variable	intermediate, variable	tends to be thicker	thicker
Canine eruption	males with delayed canine eruption	indeterminate	lacks delayed canine eruption	lacks delayed canine eruption	lacks delayed canine eruption
Premolar to molar wear gradient	slow P3 wear	indeterminate	slow P3 wear	increase of apical P3 wear	increase of apical P3 wear
Postcranial characters	Chimp/human LCA (INFERRED)	<i>Ar. kadabba/Sa. tchadensis/O. tugenensis</i>	<i>Ar. ramidus</i>	<i>Au. ananensis</i>	<i>Au. afarensis</i>
Iliac isthmus	superoinferiorly long	indeterminate	short	indeterminate	short
Pubic symphysis outline	superoinferiorly long	indeterminate	short	indeterminate	short
Ilium/iliac isthmus orientation	coronal	indeterminate	sagittal	indeterminate	sagittal
Iliac breadth	moderately broad	indeterminate	slightly broadened	indeterminate	further broadened with expanded sciatic notch
Anterior inferior iliac spine	not developed	indeterminate	strong, formed by separate ossification center	indeterminate	strong, formed by separate ossification center
Public ramus	mediolaterally short	indeterminate	mediolaterally short	indeterminate	elongated
Ischium	long	indeterminate	long	indeterminate	abbreviated
Ischial tuberosity	not angulated	indeterminate	not angulated (INFERRED)	indeterminate	angulated
Greater sciatic notch	not developed	indeterminate	weak	indeterminate	well-developed
Femoral hypotrochanteric fossa	lacks true fossa	lacks true fossa	lacks true fossa	intermediate?	true fossa
Third trochanter and gluteal ridge	strong/rugose 3rd trochanter leading to laterally placed gluteal line	strong/rugose 3rd trochanter leading to laterally placed gluteal line	3rd trochanter weaker but same pattern	3rd trochanter weaker but same pattern	3rd trochanter localized, gluteal line angles medially
Femoral linea aspera	widely spaced med and lat lips	widely spaced med and lat lips	widely spaced med and lat lips	widely spaced med and lat lips	usually true linea aspera
Femoral neck cortical distribution	superior cortex relatively thick	superior cortex relatively thick	indeterminate	indeterminate	superior cortex relatively thin
Hallux	fully abductable, no dorsal doming	indeterminate	fully abductable, no dorsal doming	indeterminate	permanent adduction of hallux, dorsal doming
Second metatarsal	not robust	indeterminate	shaft and base robust	indeterminate	secondary gracilization
Metatarsal heads (rays 2–5)	limited dorsal doming	indeterminate	dorsally domed (M13 known)	indeterminate	dorsally domed
Proximal foot phalangeal cant	proximal orientation	indeterminate	upwardly canted	indeterminate	upwardly canted orientation
Trapezoid	mediolaterally narrow	indeterminate	mediolaterally narrow	indeterminate	broad
Capitate	head located palmarly	indeterminate	head located palmarly	head dorsalized and broader	head dorsalized and broader
Metacarpal heads	moderate dorsal constriction	indeterminate	weak, but constriction still seen	indeterminate	constriction lacking
Metacarpal distal end	moderate/strong proximal collateral ligament facets	indeterminate	intermediate?	indeterminate	weak collateral ligament grooves
Skeletal size dimorphism	weak	indeterminate	weak	indeterminate	moderate
Megadontia relative to body size	weak	indeterminate	weak	expressed (INFERRED)	distinct

Hominid clade

LCA

Key:

Primitive condition

Intermediate derived condition

Derived condition

Comparatively few features of *Ar. ramidus* are derived relative to these earlier hominids, although many body parts of the latter are still unrepresented. There are no apparent features sufficiently unique to warrant the exclusion of *Ar.*

ramidus as being ancestral to *Australopithecus* (74), and a greatly expanded set of shared derived characters now links *Ar. ramidus* with later members of the hominid clade. Table 1 identifies some of the most important. This pattern robustly falsi-

fies earlier assessments that the Aramis fossils represent an ancestral chimpanzee (13, 75). These results are suggestive of a cohesive hominid evolutionary grade preceding *Australopithecus* (currently >6.0 to 4.2 Ma). By priority, the name *Ardipithecus* may encompass other named genera at this adaptive plateau (12, 15).

The question of whether *Ar. ramidus* is ancestral to later hominids is moot for some cladists because they consider ancestors inherently unrecognizable and therefore recognize only sister taxa (76). The fossils reported here make it even more obvious that *Ar. ramidus* is the cladistic sister to *Australopithecus/Homo* because it shares primitive characters with earlier hominids and apes but at the same time exhibits many important derived characters that are shared exclusively only with later hominids (Table 1).

Species-level phylogenetics are more difficult to discern given the sparse geographic and temporal distribution of available fossils (Fig. 5). Primitive characters seen in *Ar. ramidus* persist most markedly in its apparent (but still poorly sampled) sister species *Au. anamensis* and, to a lesser degree, in *Au. afarensis*. The known dental and mandibular remains of *Au. anamensis* are temporally and morphologically intermediate between those of *Ar. ramidus* and *Au. afarensis*, with variation that overlaps in both directions. Its constellation of primitive and derived features of the mandible, CP₃ complex, lower dm₁ (lower first deciduous molar), and postcanine dentition lends support to the hypothesis of an evolutionary sequence of *Ar. ramidus* → *Au. anamensis* → *Au. afarensis* (7, 8, 77). Circumstantial evidence supporting this hypothesis is the temporal and geographic position of *Ar. ramidus* directly below the first known appearance of *Au. anamensis* within the Middle Awash succession. Here, these taxa are stratigraphically superimposed in the same succession, separated by ~80 vertical meters representing ~200,000 to 300,000 years (7). *Au. afarensis* appears later in the same sequence [3.4 Ma, at Maka (53)].

Therefore, at one end of a spectrum of phylogenetic possibilities, *Ar. ramidus* may have been directly ancestral to the more derived chronospecies pair *Au. anamensis* → *Au. afarensis* across the full (still unknown, presumably African) species range (7, 8, 77) (Fig. 5A). Although *Au. afarensis* is well represented in craniodental remains and postcrania, its apparent earlier chronospecies *Au. anamensis* is still woefully underrepresented in both, and because *Ar. ramidus* is so far known only from limited time horizons and locations, its last appearance, date, and potential relationship to these later taxa are still indeterminate. Given the dramatic differences in postcranial anatomy seen in later *Australopithecus* and hinted at in known *Au. anamensis*, it seems likely that a major adaptive shift marked the *Ardipithecus*-to-*Australopithecus* transition (when- ever and wherever the transition might have occurred and whatever its population dynamics). This transition may not have occurred through

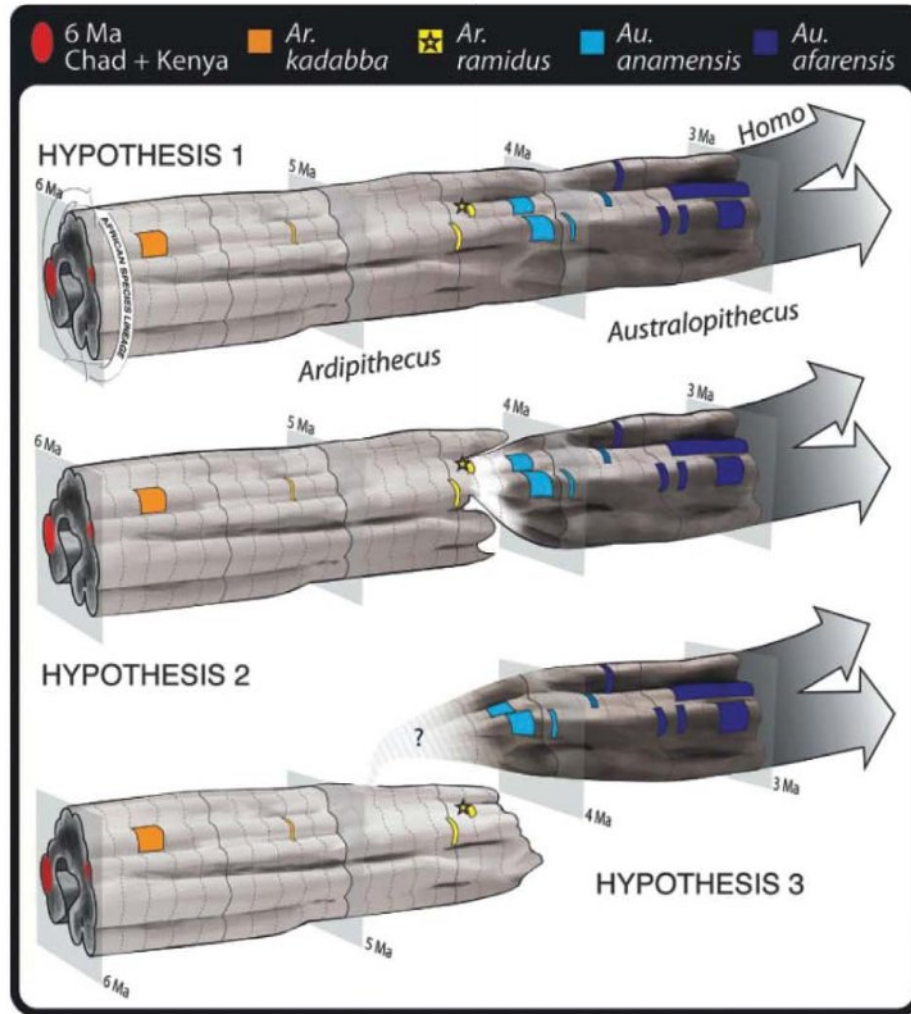


Fig. 5. Geographic and temporal sparsity of early hominid fossils. Colored windows represent presently available samples. Specific and subspecific relationships are currently impossible to resolve because of limited available data. Depicted species lineages are currently impossible to resolve because of limited available data. Depicted species lineages are currently impossible to resolve because of limited available data. Depicted species lineages are currently impossible to resolve because of limited available data. The slice at ~6 Ma reveals the two known (red) samples of Late Miocene hominids (Chad and Kenya), schematized here for simplicity within the same bundle, pending additional evidence (12). *Au. afarensis* is (so far) sampled in the Ethiopian, Kenyan, Tanzanian, and Chadian (hidden behind the bundle) regions. The Ethiopian Afar region has yielded four named, time-successive taxa, including *Ar. ramidus* (yellow star). The close chronological and geographic proximity of *Ar. ramidus* and *Au. anamensis* within the Middle Awash stratigraphic succession can be accommodated in different stratophenetic arrangements, each with different predictions about future fossil discoveries. Hypothesis 1 interprets all known evidence to represent a species lineage evolving phylogenetically across its entire range. Hypothesis 2 depicts the same evidence in an *Ardipithecus*-to-*Australopithecus* transition (speciation) occurring between ~4.5 and ~4.2 Ma in a regional (or local) group of populations that might have included either or both the Afar and Turkana rifts. Hypothesis 3 accommodates the same evidence to an alternative, much earlier peripheral allopatric "rectangular" speciation model (cladogenesis through microevolution accumulated in a peripheral isolate population, becoming reproductively separated). Other possibilities exist, but at the present time, none of these hypotheses can be falsified based on the available evidence. To choose among them will require more fossil evidence, including well-documented transitions in multiple geographic locales. See the text [and (7)] for details.

pan-specific phyletic evolution (Fig. 5A). Figure 5 presents two other phylogenetic hypotheses that are also, at present, impossible to falsify.

If diagnostic contemporary fossils of *Au. anamensis* are someday found in rocks of >4.4 Ma, the hypothesis that the Afar population of *Ar. ramidus* is the phyletic ancestor of *Au. anamensis* (Fig. 5A, B) would be falsified. In such an eventuality, Aramis *Ar. ramidus* would represent a persisting relict population of the mother species (Fig. 5C). Given the lack of relevant fossils, it is currently impossible to determine whether there was a geologically rapid phyletic transition between *Ardipithecus* and *Australopithecus* in the Middle Awash or elsewhere. Nevertheless, the morphological and ecological transition between these two adaptive plateaus is now discernible.

***Ardipithecus* and *Australopithecus*.** For Darwin and Huxley, the basic order in which human anatomies, physiologies, and behaviors were assembled through time was unknown—indeed unknowable—without an adequate fossil record. They were forced to employ extant ape proxies instead. The latter are now shown to be derived in ways unrelated to the evolution of hominids.

The Aramis fossils help clarify the origin of the hominid clade (27, 31), and reveal some paleobiological dimensions of the first hominid adaptive plateau (*Ardipithecus*). The primitive characters of *Ar. ramidus* simultaneously provide a new perspective on the evolutionary novelties of *Australopithecus*.

Even in the wake of the Aramis and Gona discoveries, the morphological envelopes, phylogenetic relationships, and evolutionary dynamics of early hominid species remain incompletely understood (Fig. 5). However, the paleobiology of *Ar. ramidus*—even when viewed through its geographically and temporally restricted Afar samples—now reveals that the basal hominid adaptive plateau comprised facultatively bipedal primates with small brains, reduced nonhoning canines, unspecialized postcanine dentitions, and arboreally competent limb skeletons. Their ecological niche(s) were probably more restricted—and their geographic distribution(s) possibly smaller and more disjunct—than those of later hominid species and genera.

The derived postcranial elements of *Australopithecus* provide a strong contrast to their more primitive homologs in *Ardipithecus* (78). Relative to the generalized anatomy of the latter, the highly evolved specializations of the foot, ankle, knee, pelvis, wrist, and hand of *Au. afarensis* (79–81) indicate that this species lineage had largely abandoned locomotion in the arboreal canopy (and its resources).

Given the strong selection predicted to have been associated with the emergence of new ranging and feeding patterns in *Australopithecus*, the transition from *Ardipithecus* to *Australopithecus* could have been rapid, and anatomically particularly so in hindlimb structure. The forelimb

(especially the hand) was probably under less intensive selection. It is possible that modification of general cis-regulatory pathways may have generated the striking and novel morphology of the hindlimb, especially the foot, because the autopod seems to be the most morphologically compliant to such mechanisms of modification. The dentognathic shifts could have been more gradational, whatever the mode of phylogenesis.

Homo and *Australopithecus* are the only primates with nongrasping feet, and this particular transformation was probably far-reaching, with consequences for key behavioral constancies in higher primates related to arboreal feeding and nesting. Without stabilizing selection for *Ardipithecus*-like arboreal capacities involving slow and careful climbing, the foot, pelvis, and thigh would have experienced directional selection to optimize bipedal locomotion during prolonged walking (also in more limited running bouts). With expanded ranging and social adaptations associated with terrestrial feeding in increasingly open environments, the transition could have been profound, but probably rapid, and therefore difficult to probe paleontologically.

One possible dynamic of an *Ardipithecus*-to-*Australopithecus* transition would have involved microevolution within a deme or regional group of demes. Being more ecologically flexible, the derived, potentially speciated populations would have undergone rapid range expansion, perhaps even encountering relict *Ardipithecus* populations. Unfortunately, the phylogeographic details remain obscure given the poor spatial and temporal resolution of the current fossil record (Fig. 5). This provides a strong incentive for pursuing that record by actively increasing sampling of sediments from different African basins with dates between ~5 and ~3.5 Ma.

Currently, *Australopithecus* appears relatively abruptly in the fossil record at about 4.2 Ma. Relative to *Ar. ramidus*, available early *Australopithecus* is now revealed to have been highly derived: a committed biped with slightly enlarged brain, a nongrasping arched foot, further derived canines, substantially specialized postcanine teeth with thick molar enamel, and expanded ecological tolerances and geographic ranges. It is widely recognized that this is the adaptive plateau antecedent to *Homo*, which is now definable as the third such major adaptive shift in human evolution. Commitment to the terrestrial ranging behaviors of *Australopithecus* well before the Pleistocene appear to have catalyzed the emergence of what must have been even more highly specialized social and ecological behaviors remarkably elaborated in descendant *Homo*—the ultimate global primate generalist.

Conclusions. Besides hominids, the only apes to escape post-Miocene extinction persist today as relict species, their modern distributions centered in forested refugia. The markedly primitive *Ar. ramidus* indicates that no modern ape is a realistic proxy for characterizing early hominid

evolution—whether social or locomotor—as appreciated by Huxley. Rather, *Ar. ramidus* reveals that the last common ancestor that we share with chimpanzees (CLCA) was probably a palmigrade quadrupedal arboreal climber/clamberer that lacked specializations for suspension, vertical climbing, or knuckle-walking (24–27). It probably retained a generalized incisor/postcanine dentition associated with an omnivorous/frugivorous diet less specialized than that of extant great apes (22, 23). The CLCA probably also combined moderate canine dimorphism with minimal skull and body size dimorphism (22, 23), most likely associated with relatively weak male-male agonism in a male philopatric social system (22, 23, 31).

Ardipithecus reveals the first hominid adaptive plateau after the CLCA. It combined facultative terrestrial bipedality (25, 26) in a woodland habitat (28–30) with retained arboreal capabilities inherited from the CLCA (24–27). This knowledge of *Ar. ramidus* provides us, for the first time, with the paleobiological substrate for the emergence of the subsequent *Australopithecus* and *Homo* adaptive phases of human evolution. Perhaps the most critical single implication of *Ar. ramidus* is its reaffirmation of Darwin's appreciation: Humans did not evolve from chimpanzees but rather through a series of progenitors starting from a distant common ancestor that once occupied the ancient forests of the African Miocene.

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 73. For example, it has been noted that these methods fail to accurately resolve relationships of modern hominoid species without sufficient intermediate forms from a fossil record (71).
 74. Enamel thickness of *Ar. ramidus* molars ranges largely from what would traditionally be termed “intermediate thin” to “intermediate thick” categories. Lacking the derived thickness pattern of *Pan*, it forms a suitable ancestral condition for later *Australopithecus*. The ubiquitous single-rooted lower fourth premolar (P₄) in known Aramis and Gona *Ar. ramidus* is notable, but this is also a known variation of *Au. anamensis* and *A. afarensis*. Judging from the clear dominance of double-rooted lower P₄’s in *Au. afarensis* (and thereafter an increasing robusticity of the roots themselves in *Australopithecus*), either there was selection for larger, more complex premolar root systems or such morphologies emerged as pleiotropy of postcanine enhancement.
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 78. We use genera to express both phyletic proximity and circumscribed adaptive systems, with ecobehavioral and morphological conditions being integral parts of the latter. This use employs the broadly defined genus *Australopithecus*, without recognizing the now commonly used *Paranthropus* (82). This is because both “robust” and “nonrobust” *Australopithecus* species are characterized by a commonly derived heavy masticatory apparatus (albeit to differing degrees), and also because we cannot—even to this day—be certain that the “robust” species are monophyletic.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/64/DC1

SOM Text

Tables S1 and S2

References

4 May 2009; accepted 8 September 2009

10.1126/science.1175802

Macrovertebrate Paleontology and the Pliocene Habitat of *Ardipithecus ramidus*

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A diverse assemblage of large mammals is spatially and stratigraphically associated with *Ardipithecus ramidus* at Aramis. The most common species are tragelaphine antelope and colobine monkeys. Analyses of their postcranial remains situate them in a closed habitat. Assessment of dental mesowear, microwear, and stable isotopes from these and a wider range of abundant associated larger mammals indicates that the local habitat at Aramis was predominantly woodland. The *Ar. ramidus* enamel isotope values indicate a minimal C₄ vegetation component in its diet (plants using the C₄ photosynthetic pathway), which is consistent with predominantly forest/woodland feeding. Although the Early Pliocene Afar included a range of environments, and the local environment at Aramis and its vicinity ranged from forests to wooded grasslands, the integration of available physical and biological evidence establishes *Ar. ramidus* as a denizen of the closed habitats along this continuum.

Circumscribing the ecological habitat of the earliest hominids is crucial for understanding their origins, evolution, and adaptations. Evidence integrated from a variety of independent geological and paleontological sources (1–3) help to place *Ardipithecus ramidus* in its regional and local Pliocene environmental settings. Here, we assess fossils of the larger vertebrates (mammalian families in which most species exceed 5 kg adult body weight) to reveal characteristics of their diets, water use, and habitat preferences.

At Aramis 4.4 Ma (million years ago), predominantly terrestrial plants, invertebrates, and vertebrates were buried relatively rapidly on a low-relief aggrading floodplain, away from perennially moving water capable of displacing most remains (2, 3). Collection bias was avoided by a systematic 100% collection strategy (1). Therefore, the large mammal assemblage spatially associated with *Ardipithecus* in the Lower Aramis Member allows for relatively robust and precise environmental inference compared with many other hominid-bearing occurrences.

The assemblage was carnivore-ravaged and is consequently dominated by bone and dental fragments (3). It represents an attritionally derived fauna collected between two widespread marker tuffs that are today exposed along an extended erosional arc (2, 3). The larger mammal fossil assemblage (4) comprises 3837 individually cataloged specimens assigned to 42 species (6 of them newly discovered), in 34 genera of 16 families (1, 5), across a wide body size range (Fig. 1A). Many of the sampled taxa provide evidence for the evolution of African vertebrates.

We consider ecological habitat to mean the biological and physical setting normally and regularly inhabited by a particular species. Our floral definitions follow the United Nations Educational, Scientific, and Cultural Organization (UNESCO) classification of African vegetation (6). Forests have continuous stands of trees with overlapping crowns, forming a closed, often multistory canopy 10 to 50 m high; the sparse ground layer usually lacks grasses. Forests grade into closed woodlands, which have less continuous canopies and poorly developed grass layers. Woodlands have trees with canopy heights of 8 to 20 m; their crowns cover at least 40% of the land surface but do not overlap extensively. Woodland ground layer always includes heliophilous (sun-loving, C₄) grasses, herbs/forbs, and incomplete small tree and shrub understories. Scrub woodland has a canopy height less than 8 m, intermediate between woodland and bushland. As proportions of bushes, shrubs, and grasses increase, woodlands grade into bushland/thickets or wooded grasslands.

Reconstructing the Aramis biotope. Reconstructing an ancient environment based on vertebrate macrofossils is often imprecise (7). Even assemblages from a single stratigraphic interval may sample thousands of years and thus represent artificial amalgamations of different biotopes shifting on the landscape through time. Even in a geologically isochronous assemblage, animals from different habitats may be mixed by moving water or by a moving lake or river margin. Ecological fidelity can be further biased through unsystematic paleontological recovery, for example, when only more complete, identifiable, and/or rare specimens are collected.

Consequently, most early hominid-bearing open-air fossil assemblages conflate multiple biotopes (7). Under such circumstances, it is not surprising that many Pliocene hominid habitats have been referred to as a “mosaic” or “a changing mosaic of habitats” (8). Such characterizations risk confusing noise for signal and local for regional environment, particularly for collection-biased assemblages lacking temporal and spatial resolution.

Initial assessment of the fauna associated with *Ar. ramidus* indicated “a closed, wooded” environment (9), an inference subsequently misquoted as “forest” (10). This interpretation was criticized on the basis that colobine monkeys and tragelaphine bovids might be unreliable indicators (11, 12).

Taxonomic abundance. Several aspects of Lower Aramis Member larger mammal assemblage abundance data constitute strong indicators of ancient biofacies and biotope (13). The locality-specific subassemblages are remarkably

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consistent in their taphonomy and taxonomy across the ~7 km distance from the easternmost (SAG-VP-7) to westernmost (KUS-VP-2) *Ar. ramidus* localities (3).

Contemporaneous localities between the two tuffs farther south of the modern Sagantole drainage (SAG-VP-1 and -3, at the southeastern paleotranssect pole) are relatively impoverished. They lack this diverse and abundant mammal assemblage and contain no tragelaphines, no monkeys, no fossil wood or seeds, no birds, no micromammals, and no *Ardipithecus* (table S1). Complementary structural, taphonomic, and isotopic data from localities on this pole of the paleotranssect suggest a more open landscape that supported more crocodilians, turtles, and hippopotamids, presumably associated with water-marginal settings more axial in the drainage basin (2, 3).

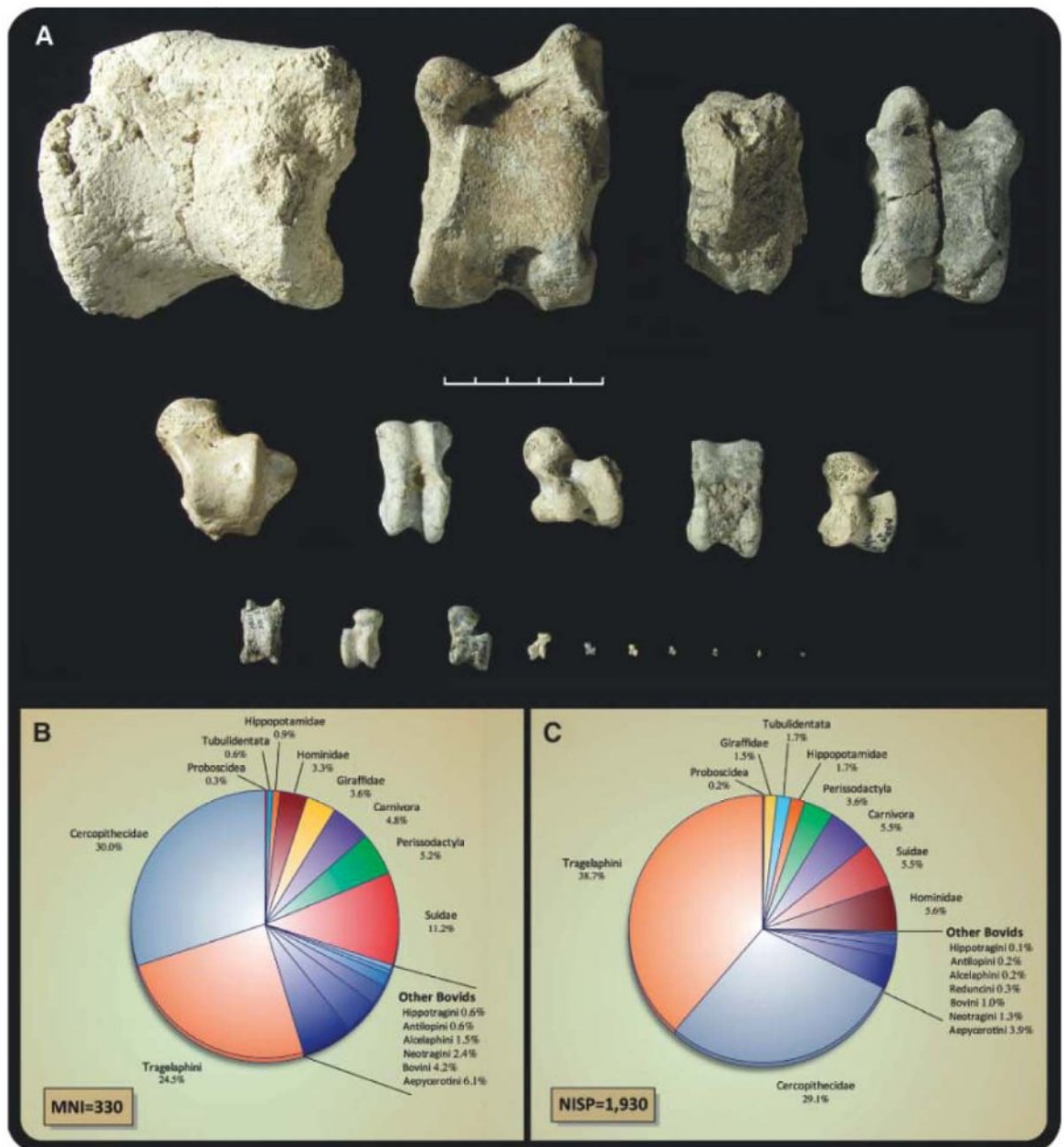
Relative and absolute abundance measures for the large mammals in our collections from the *Ardipithecus*-bearing Lower Aramis Member localities were assessed by the number of identified specimens (NISP) ($n = 1930$) and the minimum number of individuals (MNI) based on teeth ($n = 330$). Proboscideans, giraffids, and hippopotamids are rare (Fig. 1, B and C). The rhinos *Ceratotherium efficax* and *Diceros* are represented by few specimens (NISP 6 and 1, MNI 4 and 1, respectively). Unlike most other waterside Plio-Pleistocene assemblages, rhinos are more abundant than hippos at Aramis. The dental mesowear pattern and occlusal morphology of Pliocene *Ceratotherium efficax* suggest that it was predominantly a grazer but ate less abrasive forage with respect to its highly specialized Pleistocene and extant descendant *Ceratotherium simum*. The

morphological and functional properties of the recovered *Diceros* sp. molars are similar to those of the extant browsing *Diceros bicornis*.

Equids are rare. One, *Eurygnathohippus* sp. nov., is distinguished by its distal limb, which is adapted to open-country running. Its elongate-narrow snout with parabolic symphysis suggests adaptation to selective feeding. The teeth of this equid bear a low-blunt cusp morphology reflecting habitual grazing. Large carnivores and aardvarks are rare, in keeping with their trophic level (as in most other eastern African Plio-Pleistocene assemblages).

Ardipithecus ramidus is represented at Aramis and environs by >110 cataloged specimens representing a minimum number of 36 individuals [14 by upper second molar (M^2) count] in the Lower Aramis Member. These numbers are rel-

Fig. 1. Aramis large mammals. **(A)** Size range illustrated by astragali. The Lower Aramis Member contains a wide range of mammalian taxa, illustrated by this image. Top left, Rhinocerotidae; middle left, *Ardipithecus ramidus* (ARA-VP-6/500); lower left, small bovid. Included are other artiodactyls, carnivores, and rodents. **(B)** Relative abundance of larger mammal taxa at Aramis based on dental MNI. **(C)** Dental NISP based on dental individuals whose tooth crowns are more than half complete. The NISP value reflects all collected specimens identified to the taxon and excludes bulk specimens (tooth crowns less than half complete). Associated dental specimens are counted as one. The MNI values use permanent molars segregated into upper and lower first, second, and third molars, respectively. Numbers for each taxon vary between NISP and MNI, but the relative proportions hold similar. Tragelaphine bovids and cercopithecoid monkeys dominate, accounting for more than half of the assemblage, however counted.



atively low compared with many of the other macrovertebrate fossil species we collected. This rarity is consistent with that observed for hominids in other well-known vertebrate assemblages (7). *Kuseracolobus aramisi* and *Pliopapio alemui* are ubiquitous in the assemblage, accounting for 30% of both the larger mammal NISP and MNI. The colobine is numerically dominant within nearly all of the localities, and

overall by a ratio of 1.4 to *Pliopapio* (colobinae NISP:cercopithecin NISP). It is slightly larger (12 kg female, 18 kg male) than this papionin (8.5 kg, 12 kg) based on dental regressions (14). Extant colobines exhibit strong preferences for arboreal habitats; extinct African taxa range from fully arboreal to highly terrestrial (15).

Bovids and primates, particularly tragelaphines and cercopithecids, dominate the larger mammal

assemblage based on taxonomically diagnostic craniodental elements (Fig. 1). Together, these taxa account for more than half of the larger mammal specimens, whether counted by NISP or dental MNI. Both cercopithecids and bovid assemblages appear to be attritional and were ravaged heavily by carnivores after death (3).

Bovids help illuminate the local Aramis environment of the *Ardipithecus*-bearing localities. One useful index is the relative abundance of grazing versus browsing taxa, which can indicate the presence of open or closed conditions, respectively (16–19). The most ecologically sensitive of these taxa include grazing, open-habitat tribes such as Alcelaphini and Hippotragini versus the primarily browsing Tragelaphini or the riparian-associated Reduncini. Reduncine bovids commonly dominate in African Plio-Pleistocene faunal assemblages (Fig. 2), in keeping with fluvial, swampy, or lake marginal depositional conditions.

Whether counted by NISP or dental MNI, *Tragelaphus* (whose modern congeners are associated with wooded habitats) (20) is the numerically dominant Aramis bovid, comprising 85% (NISP) of the bovid assemblage (Fig. 1), followed by *Aepyceros* (whose modern form favors grassy woodland to wooded grassland environments). In contrast, alcelaphine and reduncine bovids that are plentiful at other Plio-Pleistocene sites are rare at Aramis, accounting for a mere 1% (NISP) and 4% (MNI) of all bovids. Aramis is unlike any other known African fossil assemblages in that *Tragelaphus* dominates the ungulates. (20–23) (Fig. 2).

Alcelaphines and reduncines were found at slightly higher frequencies at locality SAG-VP-7 at the eastern end of the *Ardipithecus* distribution (although tragelaphines and aepycerines still dominate there). This subtle difference between SAG-VP-7 and other more westerly hominid-bearing localities is also indicated by cercopithecids abundance. SAG-VP-7 has relatively fewer cercopithecids and more alcelaphine and reduncine bovids (Fig. 2), potentially signaling that this easternmost *Ardipithecus* locality was a transition zone between two biotopes.

Functional morphology. Taxon-based approaches to the inference of paleohabitats are usually restricted to using identifiable craniodental remains and assume that habitat preference persists through evolutionary time. Another approach is to evaluate the anatomy of fossils with respect to its implications for functional adaptations. These methods presume that mammals exhibit skeletal and dental adaptations for locomotion and feeding that correlate with their preferred environment (24). Samples of extant taxa are used to quantify the relations between skeletal/dental traits and environmental variables, with the results then applied to fossil forms (25).

Here, we evaluate the “ecomorphology” of the most common large mammals at Aramis, the bovids and cercopithecids monkeys. For the Aramis bovids, we evaluated the astragali and

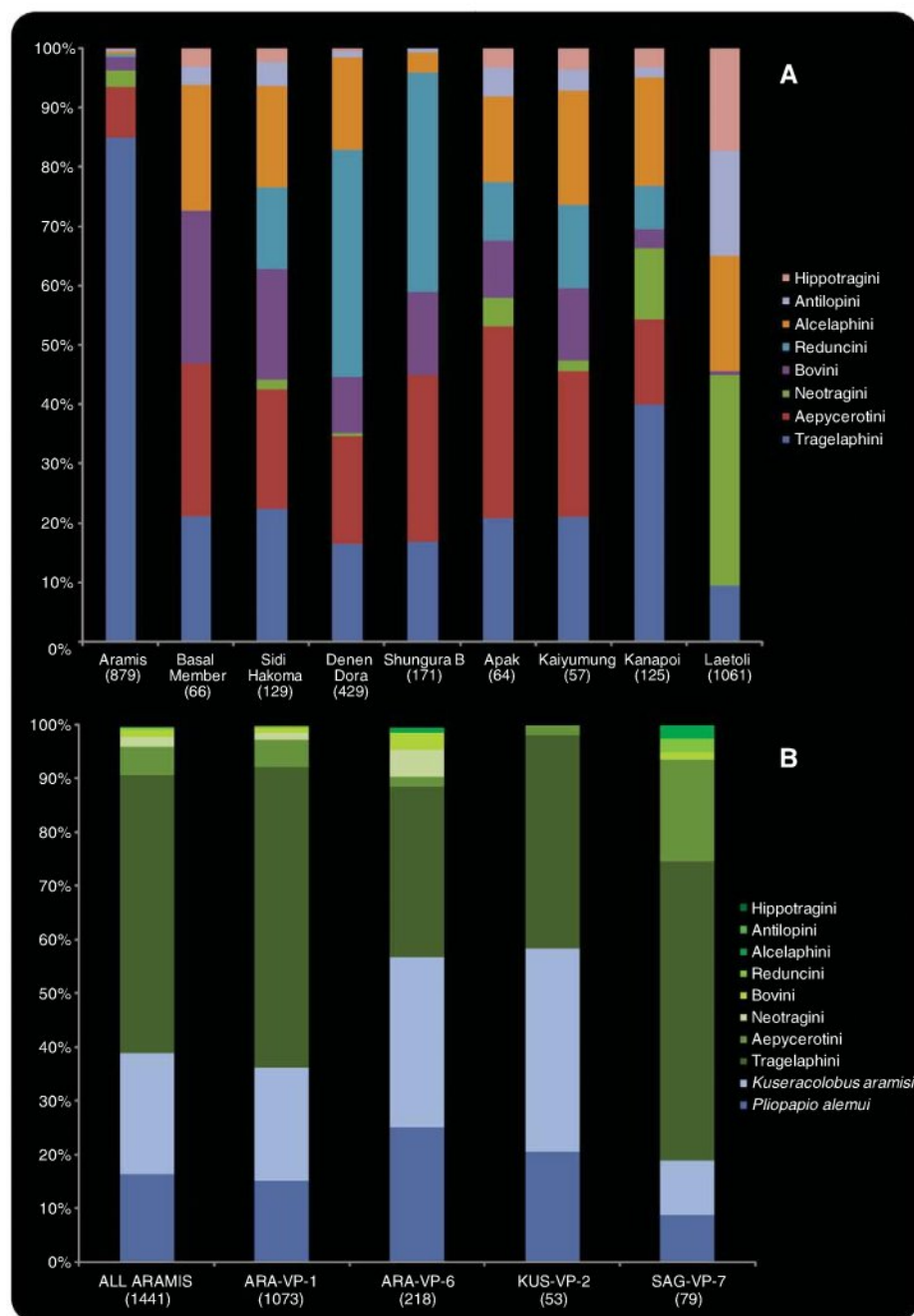


Fig. 2. Aramis taxonomic abundance. (A) Comparison between the relative abundance (dental) of bovid taxa at Aramis and other Plio-Pleistocene sites (21, 23, 45). The bovid fauna at Aramis is markedly different due to the dominance of tragelaphines. All frequencies are based on NISP, except for Hadar, which is based on MNI. (B) Within-site comparison of the relative abundances of bovids and cercopithecids. Among Lower Aramis Member localities, SAG-VP-7 has relatively lower abundances of cercopithecids and higher abundances of alcelaphines and reduncines, potentially indicative of ecotonal conditions at this easternmost locality of the *Ardipithecus* distribution.

phalanges (25, 26) because other elements that can be revealing (metapodials and femora) were not preserved in sufficient numbers. We used a four-habitat grouping scheme (26) (SOM text S1). Of the 11 available intact bovid astragali with statistically significant habitat predictions (accuracy >95%), 10 are classified as “forest” and one as “heavy cover.” This is a clear signal, since these methods typically produce more varied habitat predictions when applied to fossil samples (27, 28). To lessen possible biases introduced by confining the analysis to specimens sufficiently complete for measurement, we also examined nonmetric traits of the phalanges and classified the entire astragali/phalangeal sample by morphotype (SOM text S1, tables S2 and S3, and fig. S1). These results independently support the conclusion from metric prediction that these animals inhabited a “forest” (in the analytical, not floral, sense).

As with bovids, cercopithecoid postcranial features are routinely posited to indicate locomotion (29–31). However, systematic studies of large samples of extant taxa are generally lacking. We therefore consider most proposed correlations between cercopithecoid anatomy and locomotor mode to be of unknown reliability, pending additional study. Even so, the elbow is clearly a key joint for distinguishing between arboreal and terrestrial primate locomotion. Of 10 available Aramis cercopithecoid distal humeri, 9 are clearly consistent with “arboreal” substrate, whereas only one is consistent with “terrestrial” substrate based on current criteria. Of 9 proximal ulnae, all are

arboreal. There was no clear evidence of terrestrial adaptation in 18 proximal radii. Hence, based on current criteria, there is clear evidence of arboreal locomotor adaptations, and a paucity of terrestrial indicators, in the overwhelming majority of the Aramis cercopithecoid postcranial sample (SOM text S2).

Dental wear. The morphology, occlusal wear, and stable isotope composition of dental remains also reveal the diet—and, indirectly, habitat preferences—of some Aramis mammals. Differences in mesowear can distinguish among extant browsers, grazers, and mixed feeders (32). The Aramis neotragines, *Giraffa*, and *Tragelaphus* cluster with extant browsers (Fig. 3 and table S3), whereas *Aepyceros* falls between extant mixed feeders and nonextreme grazers. Rare Aramis alcelaphines cluster with nonextreme grazers, whereas the rare bovine and equid fossils are closest to extant coarser grass grazers.

The high cusps and colobine-like morphology of *Pliopapio alemui* (tall molars with high relief and little basal flare) suggest that the two Aramis monkey taxa had similar diets. We sampled a mixed set of colobine and cercopithecine molars for a blind test of microwear. No significant differences were found between the two taxa. Microwear on the Aramis monkey molars is consistent with both frugivory and folivory but not hard object feeding. A diet of soft (but perhaps tough) foods would be typical of colobines, and the same may have been the case for the papionin (33).

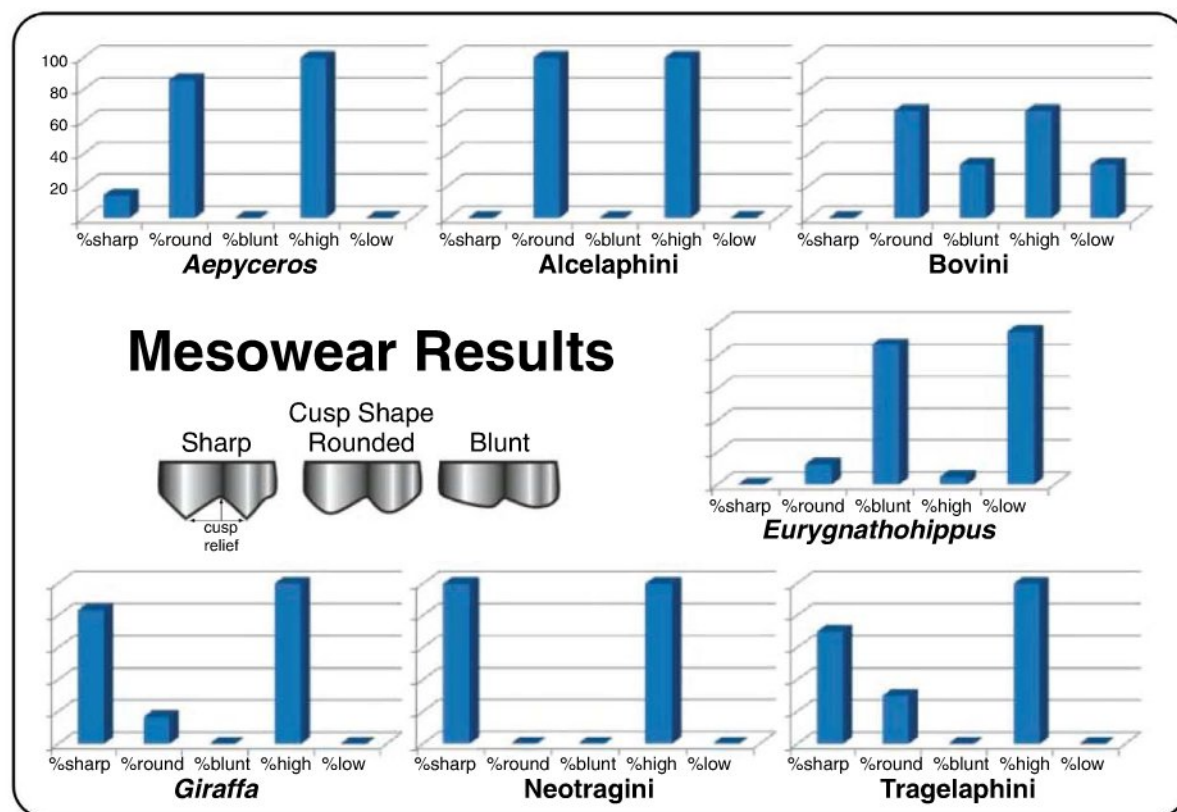
Enamel isotopes. The carbon isotopic composition of a mammal’s tooth enamel reflects the

relative contributions of grass, trees, and shrubs to its diets. Oxygen isotopes can reveal the degree that a species lives in, or consumes, water from different sources (34). We sampled tooth enamel bioapatite from 177 specimens encompassing a wide range of mammalian taxa within the *Ar. ramidus*-bearing unit (Fig. 4, SOM text S3, and table S4). These were analyzed blind to taxon. Carbon isotopic ratios for grazers are high, whereas those for mixed feeders, browsers, and forest floor feeders decrease systematically (SOM text S4). Oxygen isotope values are low for water-dependent species such as carnivores and hippos in wet riparian habitats and higher for water-independent browsers and open dry-habitat species.

In the *Ardipithecus*-bearing Lower Aramis Member assemblage, the aquatic carnivore *Enhydriodon* (an otter) has the lowest $\delta^{18}\text{O}$ of all species. Conversely, the ursid *Agriotherium* (a bear) has the highest carnivore $\delta^{18}\text{O}$, consistent with anatomical evidence for an omnivorous diet (35). Among herbivores, giraffids (*Giraffa* and *Sivatherium*) have the highest $\delta^{18}\text{O}$ and lowest $\delta^{13}\text{C}$ values, whereas grazing equids (*Eurygnathohippus*), alcelaphines, bovines, hipopotragines, and rhinocerotids show the converse. Among primates, *Kuseracolobus* has higher $\delta^{18}\text{O}$ and lower $\delta^{13}\text{C}$ than *Pliopapio*, which resembles the difference between modern folivorous Colobini and more omnivorous Papionini (36, 37).

The carbon isotopic composition of four of five *Ardipithecus ramidus* individuals is close to

Fig. 3. Mesowear analysis results for the second molar paracone apex of fossil ungulates. Cusp shape was scored qualitatively as sharp, rounded, or blunt. The relative difference in height between tooth cusp apices and intercusp valleys (occlusal relief) was qualitatively scored as either high or low (large or small distance between cusp apex and intercusp valley, respectively). Histograms show the results on the mesowear variables measured (i.e., the percentages of sharp versus rounded versus blunt cusp shapes and the percentages of high versus low occlusal relief).



that of *Pliopapio*, reflecting diets that included small amounts of ^{13}C -enriched plants and/or animals that fed on such plants. *Ardipithecus* consumed slightly more of these resources than modern savanna woodland chimpanzees (38) but substantially less than later Plio-Pleistocene hominids (39, 40). The fifth individual has a $\delta^{13}\text{C}$ value of -8.5 per mil (‰), which is closer to, though still lower than, the means for *Australopithecus africanus*, *Au. robustus*, and early *Homo* (39, 40). Slightly lower $\delta^{18}\text{O}$ compared with *Pliopapio* and *Kuseracolobus* suggests that *Ardipithecus* obtained more water from fruits, bulbs, tubers, animals, and/or surface sources.

The isotopic composition of the Aramis mammals between the two tuffs (Fig. 4 and table S4) conforms broadly to patterns expected for their modern congeners across the forest-woodland-savanna spectrum (37, 38) in the East African rift and is consistent with other early Pliocene assemblages (39, 40). Relatively low primate, giraffid, tragelaphine, and *Deinotherium* $\delta^{13}\text{C}$ values indicate that small patches of closed canopy forests were present, although woodlands to wooded grasslands probably dominated. Low $\delta^{13}\text{C}$ values for hyaenids suggest that browsing

prey contributed more to their diet compared to their modern congeners in grazer-dominated open savanna environments (37). This is congruent with the numerical dominance of browsing tragelaphines and accords with other evidence for the dominance of woodlands in the 4.4 Ma local environment occupied by *Ardipithecus* (2, 3). A small number of rare grazing species—mainly equids, alcelaphines, hippotragines, and some impala, rhino, and bovines—have high $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, indicating that they fed on water-stressed C_4 plants in drier, open environments (41). These taxa comprise a small portion of the overall assemblage.

The large range of $\delta^{18}\text{O}$, particularly the large difference (9.6‰) between water-independent (evaporation-sensitive) Giraffidae (*Giraffa* and *Sivatherium*) and water-dependent (evaporation-insensitive) Hippopotamidae, suggests a mean annual evaporative water deficit of ~ 1500 mm (41). Therefore, Aramis was a generally dry woodland setting far from riparian environments. Enamel isotopes of these taxa from nearby pencontemporary sites at Gona (42) (SOM text S3 and fig. S2) have a $\delta^{18}\text{O}$ difference of only 4.6‰, reflecting an annual water deficit of ~ 500 mm

(41). Consistently lower oxygen isotope ratios support geological evidence that Gona was close to permanent water (43), but higher carbon isotope ratios for all Gona browsers are inconsistent with greater water availability (SOM text S3).

Other ecological approaches. An approach to deducing ancient environment is to first assign each mammal taxon in a fossil assemblage to an ecological category (usually based on diet and locomotion) and then compare the proportions of these categories in the fossil sample to a range of similarly categorized extant communities (44, 45). This approach uses only the presence or absence of taxa, so it is subject to taxonomic and taphonomic biases involving small samples and mixing. Furthermore, the results are often of low resolution because biased local fossil assemblages are compared to variably recorded modern communities that pool multiple habitats (21). *Ardipithecus ramidus* was previously interpreted as inhabiting a woodland or dry forest based on a preliminary Aramis faunal list (about 10% of the sample now available) (46). Although the full faunal list produces results consistent with this finding, these results are not highly robust because the data broadly overlap among distinct environments (e.g., open, riparian, medium-density, and closed woodland) (47).

Other measures of abundance also provide information on the trophic structure of mammalian community represented by the *Ardipithecus*-bearing Lower Aramis Member. Although there are many grazing and carnivorous species (Fig. 5), these taxa are rare (48), so a strict presence/absence evaluation distorts the ecological signal. When measures of relative abundance (NISP and MNI) are included, along with direct information on trophic levels from the stable isotope and mesowear results, a different picture emerges.

These combined data show that the large mammal biomass at Aramis was dominated by browsers and frugivores (including frugivorous animals that consume leaves as a substantial part of their diet). It is unlikely that a plethora of mammals dependent on browse and fruit would have been able to subsist in an environment without abundant trees, the presence of which is witnessed by fossil pollen as well as abundant seeds, wood, phytoliths, and rhizoliths (2).

Hominid habitat. Establishing habitat (as opposed to general environment) is crucial for illuminating the paleobiology of any fossil species, including hominids. On the basis of mixed fossil faunas, it has been previously proposed that “early hominids were apparently not restricted to a narrow range of habitats.” [(8), p. 571]. However, this raises the question of whether the hominids actually occupied a wide range of habitats or whether taphonomic processes and sampling biases have mixed hominid remains with those of species from biotopes that hominids rarely, if ever, frequented. Many fossil assemblages simply do not preserve the necessary temporal and spatial resolution needed to determine whether hominids preferred the riverine forest, lake margin,

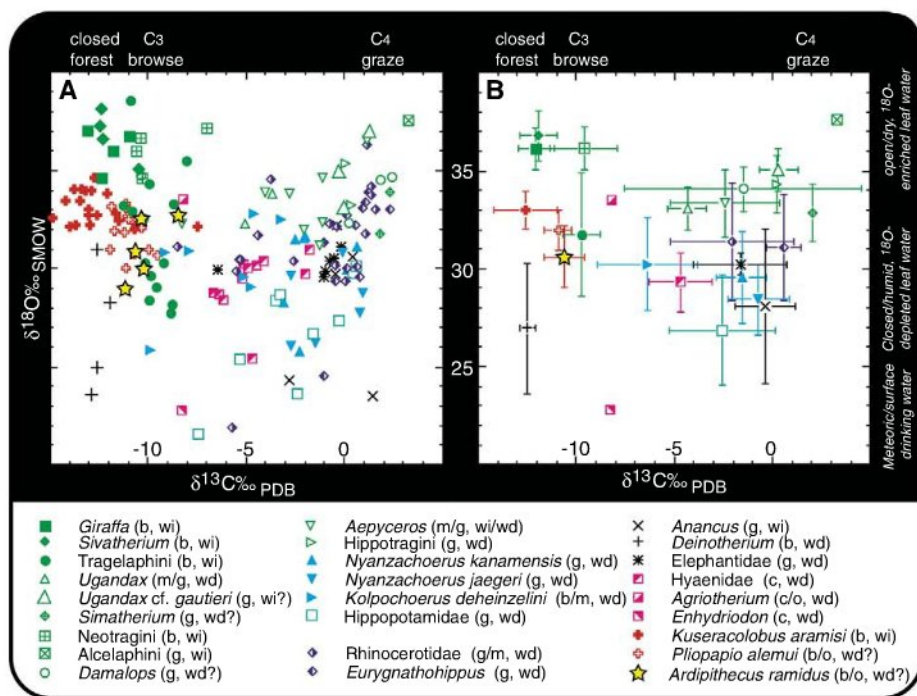


Fig. 4. Carbon and oxygen isotopic composition of mammal tooth enamel from the Lower Aramis Member of the Sagantole Formation in the Middle Awash Valley. (A) Individual $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values plotted by taxon. (B) Bivariate means ± 1 SD. See SOM text S3 for methods and interpretations, table S3 for raw data and statistics, and fig. S1 for comparison with species also occurring in roughly contemporaneous deposits at Gona (42). Food and drinking habits are inferred from closest living relatives and from carbon and oxygen isotope ratios. b, browser (C_3 feeder); m, mixed grazer/browser (C_3 and C_4 feeder); g, grazer (mainly C_4 grasses); wi, water-independent (evaporation-sensitive) (41) or obtaining substantial amounts of water from green leaves; wd, water-dependent (evaporation-insensitive) (41), relying on drinking water when plant leaves are dry; c, carnivore; o, omnivore, including diets with leaves fruit, tubers, roots, flowers (all predominantly C_3), seeds, fungi, and vertebrate and invertebrate animal matter. Diets, water use, and habitat preferences of species of extinct genera and families are indicated in italics because they are more intrinsically uncertain. Interpretations are described and justified in detail in SOM text S3.

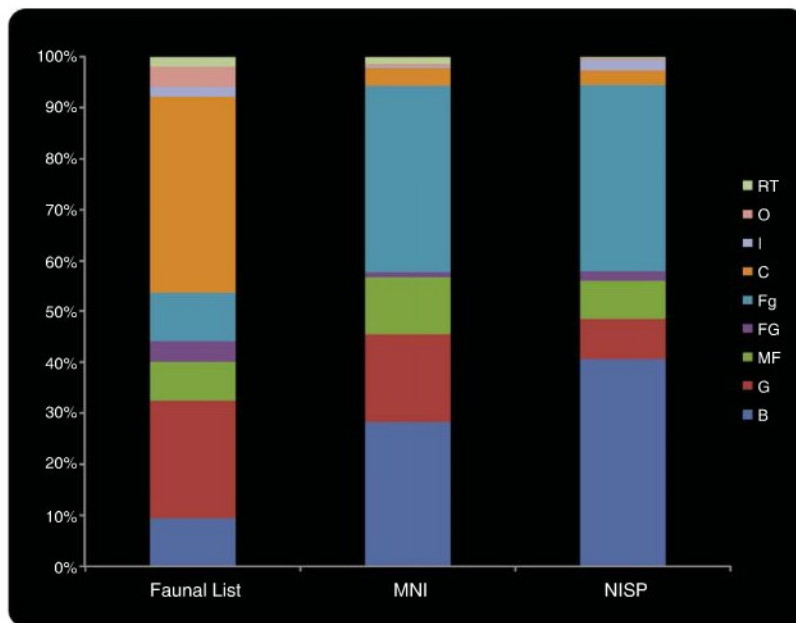


Fig. 5. Trophic ecovariable distributions by faunal list, dental NISP, and dental MNI. Comparisons of the Aramis trophic structure based on the faunal list versus specimen-level, dental relative abundance data as measured by NISP and MNI. Grazing and carnivorous species are abundant in the faunal list-based trophic structure, whereas browsers and frugivores dominate when NISP and MNI data are incorporated. B, browser; G, grazer; MF, mixed feeder; FG, fresh grass grazer; Fg, Frugivore (includes fruit and leaves); C, carnivorous; I, insectivorous; O, omnivorous; RT, root and tuber.

bushland, savanna, and/or woodland habitats demonstrably available within a few kilometers of most depositional loci within rift valley settings.

For example, *Ardipithecus ramidus* has also been found at Gona, about 70 km to the north of Aramis, in a valley margin environment where lake deposits interfingered with small fluvial channels or lapped onto basaltic cones and flows (43). At Gona, the dominance of C₃ plants indicated by paleosol isotopes contrasts with the C₄ plant signal in many associated ungulate grazers (indicated by enamel isotopic data). Levin *et al.* thus concluded that *Ardipithecus* "...may have inhabited a variety of landscapes and was not as ecologically restricted as previous studies suggest" [(42), p. 232]. The Gona paleontological and isotopic data show only that a range of habitats was present, and the attribution of *Ardipithecus* to any particular set of the available biotopes is problematical in this mixed assemblage (49). Fish, birds, browsers, horses, and hominids are all frequently found in a single mixed fossil assemblage in a fluvial or near-shore deposit. This does not mean that the fish were arboreal or that horses were aquatic. Neither do such data mean that the hominids exploited all potentially available habitats.

The Lower Aramis Member deposits provide fossil samples that evidence a range of environments in the region at 4.4 Ma (2, 3). However, the consistent association of *Ar. ramidus* with a particular fauna and flora in deposits between SAG-VP-7 and KUS-VP-2 suggests its persistent occupation of a woodland with patches of forest across the paleolandscape (2, 3). *Ardipithecus*

has not been found in the apparently more open settings to the southeast. There is no evidence of any taphonomic bias related to *Ardipithecus* that might produce this pattern (3) and no evidence of any other spatial or stratigraphic clustering within the 4.4 Ma Lower Aramis Member interval.

Based on a range of independent methods for inferring habitat-based large samples of consilient spatial, geological, and biological evidence generated from diverse sources, we therefore conclude that at Aramis, *Ar. ramidus* resided and usually died in a wooded biotope that included closed through grassy woodlands and patches of true forest [sensu (6)]. There is no evidence to associate this hominid with more open wooded grasslands or grassland savanna.

Isotopic data indicate that the *Ar. ramidus* diet was predominantly forest- to woodland-based. This interpretation is consistent with evidence of the dental and skeletal biology of this primate (1). The ecological context of 4.4 Ma Aramis hominids, combined with their absence or extreme rarity at Late Miocene and Early Pliocene sites, suggest that the anatomy and behavior of the earliest hominids did not evolve in response to open savanna or mosaic settings. Rather, this clade appears to have originated within more closed habitats favored by these peculiar primates until the origin of *Australopithecus*, and perhaps even beyond (50).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/67/DC1

SOM Text

Figs. S1 and S2

Tables S1 to S5

References

4 May 2009; accepted 14 August 2009

10.1126/science.1175822

Paleobiological Implications of the *Ardipithecus ramidus* Dentition

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The Middle Awash *Ardipithecus ramidus* sample comprises over 145 teeth, including associated maxillary and mandibular sets. These help reveal the earliest stages of human evolution. *Ar. ramidus* lacks the postcanine megadontia of *Australopithecus*. Its molars have thinner enamel and are functionally less durable than those of *Australopithecus* but lack the derived *Pan* pattern of thin occlusal enamel associated with ripe-fruit frugivory. The *Ar. ramidus* dental morphology and wear pattern are consistent with a partially terrestrial, omnivorous/frugivorous niche. Analyses show that the ARA-VP-6/500 skeleton is female and that *Ar. ramidus* was nearly monomorphic in canine size and shape. The canine/lower third premolar complex indicates a reduction of canine size and honing capacity early in hominid evolution, possibly driven by selection targeted on the male upper canine.

Fossilized teeth typically represent the most abundant and best preserved remains of hominids and other primates. They provide crucial evidence on variation, phylogenetic relationships, development, and dietary adaptations. Furthermore, because canines function as weapons in interindividual aggression in most anthropoid species, they additionally inform aspects of social structure and behavior.

We have now recovered and analyzed a sample of 145 non-antimeric tooth crowns comprising 62 cataloged dentition-bearing specimens of *Ardipithecus ramidus* from the Lower Aramis Member of the Sagantole Formation, about five times more than previously reported (1, 2) (Fig. 1 and table S1). All permanent tooth positions are represented, with a minimum of 14 individuals for both the upper canine and upper second molar (M²) positions. Excluding antimeres, 101 teeth have measurable crown diameters. In addition, seven *Ar. ramidus* specimens with teeth have been described from Gona (3). These are broadly comparable to their Aramis counterparts in size, proportions, and morphology but slightly extend the smaller end of the species range in some mandibular crown diameters.

The major morphological characteristics of the *Ar. ramidus* dentition have been outlined in previous studies of Aramis and Gona fos-

sils (1, 3, 4). Comparisons of *Ar. ramidus* with Late Miocene hominids (*Ar. kadabba*, *Orrorin tugenensis*, and *Sahelanthropus tchadensis*) have identified slight but distinct differences, particularly in the canine (4–6). Other subtle features of incisors and postcanine teeth have been noted as phylogenetic or taxonomic distinctions (5–10). However, the most recent and comprehensive evaluation of the available Late Miocene materials concluded that these differences are minor compared with extant ape (and later hominid) genus-level variation and that both *Ar. ramidus* and *Ar. kadabba* dentitions exhibit phenetic similarities with early *Australopithecus* (4).

The expanded *Ar. ramidus* sample of the present study allows a more definitive phylogenetic placement of *Ar. ramidus* relative to the more primitive *Ar. kadabba* and the more derived *Au. anamensis* and *Au. afarensis* (11). Here, we focus on the paleobiological aspects of the *Ar. ramidus* dentition, including variation, size, and scaling, probable dietary niche, and canine/lower third premolar (C/P₃) complex evolution and its behavioral implications. We also address the alleged phylogenetic importance (7) of enamel thickness in *Ar. ramidus* (1). This is now made possible by the more comprehensive dental collection that includes key associated dental sets.

Crown size, proportions, and variation. The *Ar. ramidus* dentition is approximately chimpanzee-sized (fig. S1 and tables S2 to S4). Mean canine size is comparable to that of female *Pan troglodytes*, although the incisors are smaller. Upper and lower first molars (M1s) are *P. troglodytes*-sized but tend to be buccolingually broader (figs. S1 to S3). The second and third molars (M2s and M3s) are both absolutely and relatively larger (figs. S1 and S4 to S6). Postcanine size and proportions of *Ar. ramidus* are similar to those of *Ar. kadabba* and other ~6.0-million-year-old forms (*O. tugenensis* and *S. tchadensis*) (4–10), as well as to many Mio-

cene hominoids (although Miocene ape lower molars tend to be buccolingually narrower) (fig. S3).

Variation within the Aramis dental sample is low. In modern anthropoids, the coefficient of variation (CV) is lowest in M1 and M2, with single-sex and mixed-sex values usually ranging from about 3.5 to 6.5 (12–14). At Aramis, *Ar. ramidus* upper and lower M1s and M2s are less variable (CVs ranging from 2.5 to 5.6) than those of *Australopithecus afarensis* and *Au. anamensis* (table S2). However, these *Australopithecus* samples represent multiple sites and span a much greater time than the Aramis fossils (11). The low variation seen in Aramis *Ar. ramidus* probably reflects spatially and temporally restricted sampling and low postcanine sexual dimorphism as in *Pan* (15) (table S5).

The Aramis postcanine dentition is also morphologically more homogenous than known *Australopithecus* species samples. For example, the six relatively well-preserved M¹s (Fig. 1) differ little in features otherwise known to vary widely within hominid and modern hominoid species (16, 17), including Carabelli's expression, occlusal crest development, and hypocone lingual bulge. This suggests that the Aramis *Ar. ramidus* collection samples regional demes or local populations with persistent idiosyncratic tendencies. The ubiquitous occurrence of single rooted lower fourth premolars (P₄) (now seen in eight non-antimeric Aramis P₄s) suggests increased frequency of otherwise rare variants from genetic drift, absent substantial selection for larger and/or more complicated root systems (18). Because this anatomy is shared with Gona *Ar. ramidus* (3), it appears characteristic of this regional population.

Morphology and evolution of the C/P₃ complex. The C/P₃ complex of anthropoids has behavioral and evolutionary importance because canine size and function are directly linked to male reproductive success (19). Therefore, clarifying the tempo and mode of the evolution of the C/P₃ complex, from hominid emergence through its early evolution, is important.

Not counting antimeres, 23 upper and lower canines from 21 *Ar. ramidus* individuals are now known from Aramis. Three more have been described from Gona (3), and seven from the ~6.0-million-year-old *Ar. kadabba*, *O. tugenensis*, and *S. tchadensis* (4–10). There are no examples of a distinctly large male morphotype in any of these collections (Fig. 1 and figs. S7 and S8), suggesting that canine sexual dimorphism was minimal in Mio-Pliocene hominids. In basal crown dimensions, *Ar. ramidus* canine/postcanine size ratios overlap extensively with those of modern and Miocene female apes (fig. S9). Absolute and relative canine heights are also comparable to those of modern female apes, although canine height appears exaggerated in *P. troglodytes* [Fig. 1; figs. S8, S10, and S11; and supporting online material (SOM) text S1].

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Canine shape of *Ar. ramidus* is either comparable to female apes or more derived toward *Australopithecus* (11) (Fig. 1 and figs. S12 and

S13). The upper canine (UC) is clearly derived in *Ar. ramidus*, because it has a diamond-shaped lateral crown profile with elevated and/or flar-

ing crown shoulders ($n = 5$ from Aramis and $n = 1$ from Gona) [this study and (3, 4, 6)]. However, the lower canine (LC) retained much more of the morphology of the female ape condition (4, 5) (Fig. 1, figs. S11 to S13, and SOM text S1). A hominid-like incisiform LC morphology (high mesial shoulder, developed distal crest terminating at a distinct distal tubercle) is seen in some female apes (e.g., *Ouranopithecus* and *P. paniscus*), whereas the LCs of *Ar. kadabba* and *Ar. ramidus* tend to be conservative, exhibiting a strong distolingual ridge and faint distal crest, typical of the interlocking ape C/P₃ complex (4) (Fig. 1 and SOM text S1).

The *Ar. ramidus* P₃ is represented by seven observable crowns, ranging from obliquely elongate to transversely broad (1) (fig. S14). The *Ar. ramidus* P₃ is relatively smaller than that of *Pan* and typically not as asymmetric or elongate in occlusal view (figs. S15 and S16). In these respects, the *Ar. ramidus* P₃ is comparable to those of *Au. anamensis* and *Au. afarensis*. However, *Ar. ramidus* is more primitive than *Australopithecus* in retaining a proportionately higher P₃ crown (fig. S16). It appears that there was a decrease of P₃ size from the ancestral ape to *Ar. ramidus* conditions, but this reduction was greater in basal crown dimensions than in crown height (SOM text S1).

In *Ar. ramidus*, the combined effect of (i) reduced canine size and projection and (ii) reduced size and mesiobuccal extension of the P₃ results in the absence of upper canine honing (defined as distolingual wear of the UC against the mesiobuccal P₃ face, cutting into the lingual UC crown face and resulting in a sharpened distolabial enamel edge). Instead, apical wear in *Ar. ramidus* commences early and thereafter expands as wear progresses. None of the known UCs or P₃s exhibits evidence of honing (fig. S14). However, both upper and lower canines project beyond the postcanine occlusal plane before heavy wear, resulting in steep and beveled wear slopes, as also seen in examples of *Au. afarensis* and *Au. anamensis* (1, 4, 20).

Two *Ar. ramidus* specimens provide associated maxillary and mandibular dentitions with minimal canine wear. One is almost certainly female (ARA-VP-6/500), and the other is a probable male (ARA-VP-1/300) (see below). Both individuals possess a UC with a shorter crown height than the associated LC (>10% difference in ARA-VP-1/300) (21). In contrast in most anthropoid species, the UC is greater in height than the LC (fig. S17), a condition exaggerated in males of dimorphic species (over 50% in some papionins). Although less extreme in extant great apes (22), the UC still exceeds LC crown height by up to ~20% (fig. S18). In modest samples of modern great ape canines with little to no wear, we found no instances of LC height exceeding that of the UC (25 males and 27 females). This pattern of relative UC and LC height in *Ar. ramidus* appears unique among anthropoids and indicates differential reduction

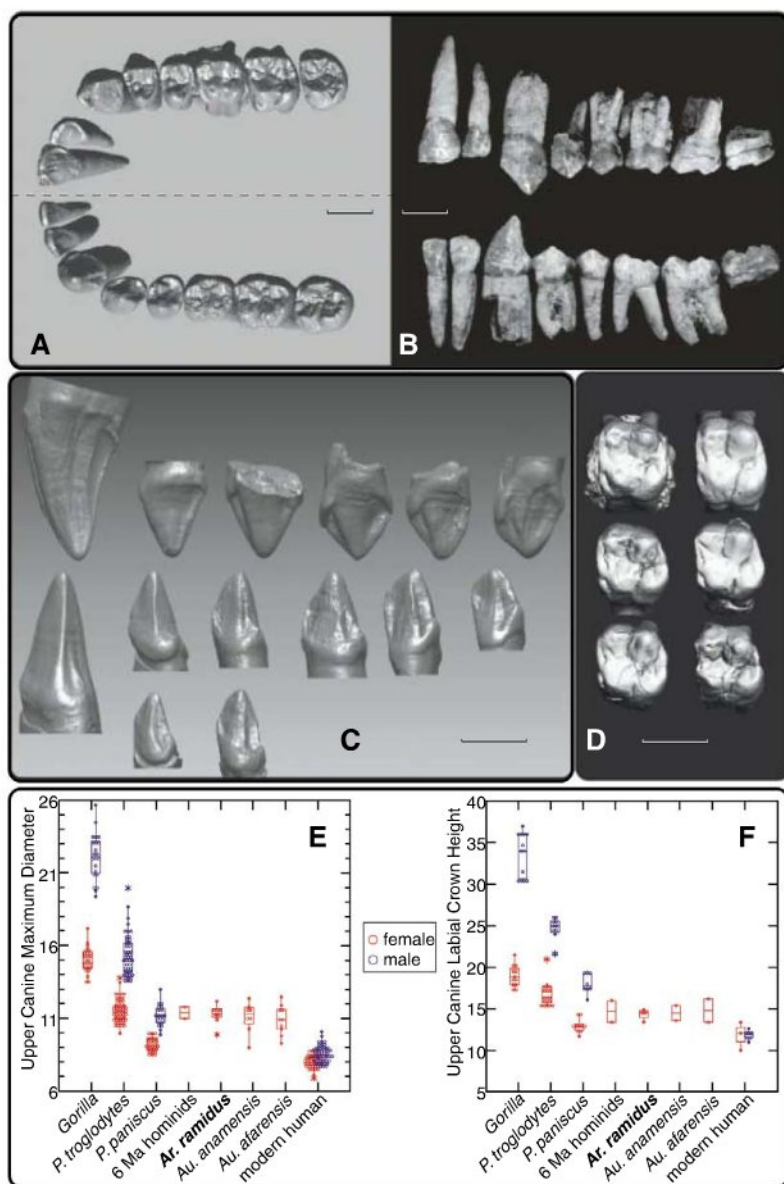


Fig. 1. Representative examples of the Aramis *Ardipithecus ramidus* dentition. (A) Occlusal view micro-CT-based alignment of ARA-VP-1/300: top, maxillary dentition; bottom, mandibular dentition. The better-preserved side was scanned and mirror-imaged to form these composites. (B) ARA-VP-1/300 in buccal view: top, right maxillary dentition (mirrored); bottom, left mandibular dentition. (C) Comparison of canine morphology (micro-CT-based renderings). Top row, lingual view of upper canines, from left to right: male *P. troglodytes* (cast), female *P. troglodytes* (cast), *Ar. kadabba* ASK-VP-3/400, *Ar. ramidus* ARA-VP-6/1, *Au. afarensis* L.H. 6 (cast), *Au. afarensis* A.L. 333x-3 (cast, mirrored). Lower rows, distolingual view of lower canines, main row from left to right: male *P. troglodytes* (cast), female *P. troglodytes* (cast), *Ar. kadabba* (STD-VP-2/61), *Ar. ramidus* ARA-VP-1/300, *Au. africanus* Sts 50 (mirrored), *Au. africanus* Sts 51. Lowest two specimens are ape lower canines with hominid-like features: left, *P. paniscus* (cast); right, *Ouranopithecus macedoniensis* RLP-55 (cast). The *Ar. ramidus* upper canine is highly derived, with a diamond-shaped crown with elevated crown shoulders. The lower canine tends to retain aspects of primitive ape features. Further details are given in the SOM figures and SOM text S1. (D) M¹ morphology (micro-CT-based renderings) showing relatively little morphological variation among the Aramis individuals. Top row left, ARA-VP-1/300 (mirrored); right, ARA-VP-1/1818. Middle row left, ARA-VP-1/3288; right, ARA-VP-6/500. Bottom row left, ARA-VP-6/502 (mirrored); right, KUS-VP-2/154. (E and F) Box plot of upper canine maximum diameter and labial height (in mm). *Ar. ramidus* includes Aramis and published Gona materials (2). The ~6-million-year-old hominids are represented by *Ar. kadabba* (ASK-VP-3/400) and *O. tugenensis* (BAR 1425'00) (7). Symbols give central 50% range (box), range (vertical line) and outliers. See SOM figures and text S1 for additional plots and details.

of the UC in hominids. The UC < LC height relation is retained in modern humans.

Morphological changes in the series *Ar. kadabba*–*Ar. ramidus*–early *Australopithecus* support the hypothesis of selection-induced UC reduction. As detailed above, the UC is clearly derived in *Ar. ramidus*, whereas the LC tends to retain the primitive female apelike condition. *Au. anamensis*, geologically younger than *Ar. ramidus* but older than *Au. afarensis*, exhibits a polymorphic condition represented by both primitive and advanced LC morphologies (4, 20) (SOM text S1). The more incisiform morphology becomes universal in *Au. afarensis* and later hominids. Furthermore, compared with both male and female apes, *Ar. ramidus* exhibits a small UC crown (both basal diameter and height) relative to apico-cervical root length, more so than the LC (figs. S19 and S20). This observation provides further support to the interpretation that the UC crown was differentially reduced (SOM text S1).

A broader comparison of *Ar. ramidus* with extant and Miocene apes illuminates aspects of C/P₃ complex evolution. Compared with cercopithecoids, hominoids tend to have smaller P₃s with less extensive honing (fig. S15). Compared with other modern and Miocene apes, both species of *Pan* appear to show P₃ reduction. The P₃ of *Ar. ramidus* is even smaller, suggesting further reduction of the C/P₃ complex from an ancestral ape condition. At first sight, the comparatively small P₃ size in *Pan* appears paradoxical, because among the modern great apes both male and female *P. troglodytes* have relatively large and tall canines (figs. S9 and S10 and SOM text S1). However, this apparent paradox is removed by a broader perspective on tooth and body size proportions. Both *Pan* species share with atelines and *Presbytis* (sensu stricto) small postcanine size relative to body size (Fig. 2, figs. S21 and S22, and SOM text S2), low postcanine dimorphism,

and low to moderate canine size dimorphism (figs. S23 to S25). Conversely, papionins exhibit the opposite condition: large postcanines, large canines, and extreme dimorphism. We therefore hypothesize that the basal *Pan* condition was characterized by a somewhat reduced C/P₃ complex as part of a generally small dentition relative to body size and that the canines were secondarily enhanced leading to modern *P. troglodytes*.

The ARA-VP-6/500 skeleton and sexual dimorphism. Of the 21 individuals with canines, ARA-VP-6/500 has UC and LC that are strikingly small; its UC ranks either 12th or 13th (of 13), and its LC ranks seventh (of eight) in size (table S6). However, postcranially, ARA-VP-6/500 is a large individual with an estimated body weight of ~50 kg (23). Was ARA-VP-6/500 a small-canined male or a large-bodied female?

We began our evaluation of ARA-VP-6/500 (24) by estimating the degree of dimorphism in the *Ar. ramidus* canine (SOM text S3). Even in modern humans, the canine is metrically the most dimorphic tooth. Mean basal crown diameter of human male canines is about 4 to 9% larger than that in females (table S5). Our analysis indicates that *Ar. ramidus* was probably only marginally more dimorphic than modern humans (tables S6 to S9 and SOM text S3), with a probable range of 10 to 15% dimorphism (in canine mean crown diameter). This is substantially less dimorphic than modern great apes, whose male canines (mean crown diameter) are larger than those of females by 19 to 47%.

On the basis of the above dimorphism estimate, the probability of a male having canines as small as those of ARA-VP-6/500 can be evaluated by bootstrapping (2). Assuming 12% dimorphism in mean canine size (table S8), the probability that ARA-VP-6/500 is a male is <0.03 (if the UC is ranked 12th of 13) or <0.005 (if ranked 13th) (table S9 and SOM text S4). We conclude that ARA-VP-6/500 is a large-bodied

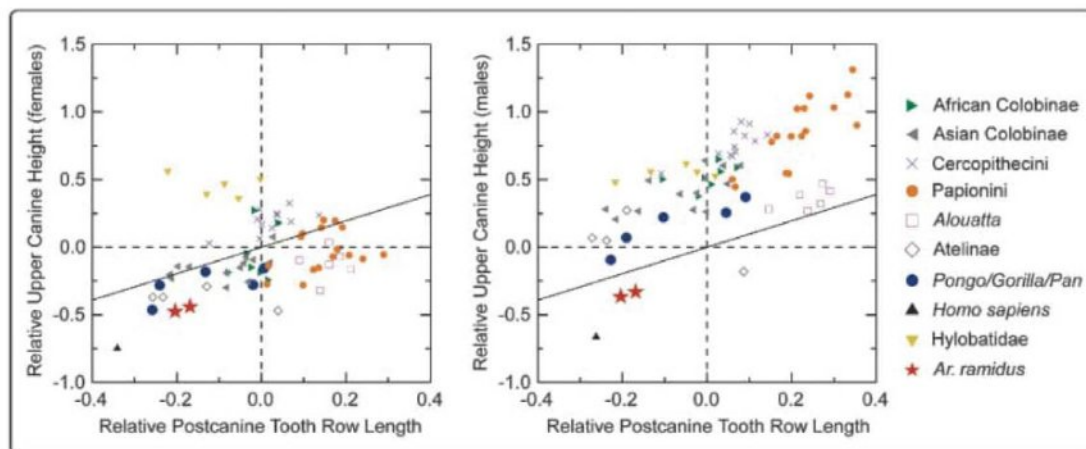
female, a conclusion also corroborated by cranial anatomy (25). This shows that skeletal size dimorphism in *Ar. ramidus* must have been slight (11), as is the case in both species of *Pan* (26, 27).

The ARA-VP-6/500 skeleton and dimorphism estimates allow us to place the *Ar. ramidus* dentition within a broader comparative framework. Scaling analyses (2) show that the UC of *Ar. ramidus* was relatively small in both sexes (fig. S22 and SOM text S2). In particular, male UC height of *Ar. ramidus* is estimated to be close to that of female *P. paniscus* and *Brachyteles* and to be much lower than that of male *P. paniscus* (which has the least projecting male canine among extant catarrhines) (Fig. 2).

Canine development and function. In cercopithecoids with highly dimorphic canines, canine eruption is typically delayed in males, beginning after the age of eruption in females (28) and apparently corresponding with species-specific patterns of body size growth spurts (29–31). Once male canine eruption is initiated, it then proceeds at a higher rate than in females, but it can still last for several years depending on species (31). As a consequence, males attain full canine eruption as they approach or achieve adult body size, both of which are necessary for reproductive success (19).

Sexually distinct patterns of canine eruption in relation to body size growth have yet to be well documented in modern great apes but appear to broadly share the cercopithecoid pattern described above (28, 32–34). Initiation of canine eruption in *P. troglodytes* differs by about 1.5 to 2 years between the sexes (35). In males of both *P. troglodytes* and *P. paniscus*, full canine eruption appears to coincide broadly with M3 eruption (observations of skeletal materials), with polymorphism in the eruption sequence of the two teeth. By contrast in females of both species, full canine eruption is attained before M3 eruption.

Fig. 2. Size and scaling of the *Ardipithecus ramidus* dentition. Natural log-log scatter diagram of relative upper canine height (y axis) against relative postcanine length (x axis): **left**, females; **right**, males. Both axes represent size free variables (residuals) derived from scaling tooth size against body size across a wide range of anthropoids (2). A value of zero represents the average female catarrhine condition. Positive and negative residuals represent relatively large and small tooth sizes, respectively. The diagonal line indicates the direction of equivalent canine and postcanine proportions independent of size. The five great ape taxa plotted are from left to right: *P. paniscus*, *P. t. troglodytes*, *P. t. schweinfurthi*, *Gorilla gorilla*, and *Pongo pygmaeus*. *Ar. ramidus* is plotted by using mean postcanine size and canine crown heights of probable female (ARA-VP-6/500) and male (ARA-VP-1/300) individuals. A hypothetical female



body weight of 45 kg or 50 kg was used (right and left stars, respectively). *Ar. ramidus* is shown to have small postcanine tooth sizes, similar to those of *Ateles*, *Presbytis* sensu stricto, and *Pan*. Relative canine height of *Ar. ramidus* is lower than that of the smallest-canined nonhuman anthropoids, *P. paniscus* and *Brachyteles arachnoides*. See SOM text S2 for further details.

The relative timing of canine eruption in *Ar. ramidus* is revealed by two juveniles. The *ARA-VP-6/1* holotype, a probable male (table S6), includes an unworn UC whose perikymata count is 193, higher than that in *Au. africanus/afarensis* (maximum 134, $n = 4$) (36) and lower than those in small samples of female *P. troglodytes* and *Gorilla* (minimum 204, $n = 10$) (37). The *ARA-VP-6/1* UC crown formation time was 4.29 or 4.82 years, depending on estimates of enamel formation periodicity (fig. S26). This is a comparatively short formation time, around the minimum reported for modern female apes (38).

The eruption pattern of a second individual, *ARA-VP-1/300*, can be assessed from the presence or absence of wear facets and/or polish. The *ARA-VP-1/300* canines were just completing eruption, its M2s were worn occlusally, and its unerupted M3 crowns were barely complete (Fig. 1 and fig. S27). Compared with extant apes, both its UC and LC development are advanced relative to M2 and M3 (fig. S28) (39).

The combined morphological and developmental evidence suggests that selection for delayed canine eruption had been relaxed in *Ar. ramidus*. We hypothesize that canine prominence had ceased to function as an important visual signal in male competitive contexts.

Tooth size and diet. We consider relative incisor and postcanine sizes to be potentially useful in inferring dietary adaptations, although consistent patterns across primates have not been obtained (40). In particular, postcanine megadontia has been considered a defining feature of *Australopithecus* (41). We evaluated incisor and molar sizes of *Ar. ramidus* in comparison to those of *Pan* and *Australopithecus*. Among anthropoids, *Pan* and *Pongo* are unique in having large incisors relative to both postcanine and body size, a condition not shared by *Ar. ramidus* (fig. S29). This suggests that *Ar. ramidus* was not as inten-

sive a frugivore as are *Pan* and *Pongo*, incisor length probably being functionally related to removal of fruit exocarp (42) and/or feeding behavior such as wadging.

Although the M1 area, normalized by individual postcranial metrics, lies well within the range of extant chimpanzees, the total postcanine area of *ARA-VP-6/500* falls between *Pongo* and *P. troglodytes* (Fig. 3). *Ar. ramidus* is not only less megadont than *Pongo* and *Au. afarensis* but, together with *Pan*, *Ateles*, and some *Presbytis* species, lies at the small end of the range of variation of large-bodied anthropoids (fig. S30). The most megadont anthropoids include robust *Australopithecus*, such as *Au. boisei*, as well as papionins and *Alouatta*. *Ouranopithecus* was probably as megadont as *Australopithecus* species, whereas *Dryopithecus* and *Pierolapithecus* probably had relative postcanine sizes closer to *Ar. ramidus* and thus better approximate the dentition-to-body size relationship of the last common ancestor of humans and chimpanzees. We conclude that *Ar. ramidus* was substantially less megadont than *Australopithecus*.

Molar structure and enamel thickness. Molar structure, enamel thickness, and tooth wear further illuminate dietary adaptation in *Ar. ramidus*. Compared with the distinct occlusal structure of the molars of each of the modern ape species (see below), *Ardipithecus* occlusal morphology is more generalized, with low, bunodont cusps and moderate to strong basal crown flare. Such morphology also characterizes *Australopithecus* as well as a diversity of Miocene apes (43). *Gorilla* molars have much more salient occlusal topography and enhanced shearing crests. *Pan* molars are characterized by broad, capacious occlusal basins flanked by moderately tall cusps, effective in crushing relatively soft, fluidal mesocarp while retaining the ability to process more fibrous herbaceous materials (Fig. 4) (44, 45). These features are ac-

centuated in *Pan* by the characteristically thin enamel of its occlusal basin (45, 46).

To further elucidate molar structure and dietary adaptations of *Ar. ramidus*, particularly in comparison with *Pan* and *Australopithecus*, we used micro-computed tomography (micro-CT) to study molar enamel thickness and underlying crown structures (2). Although the weak contrast of fossil enamel and dentin makes micro-CT-based evaluations difficult, we were able to assess several *Ar. ramidus* molars with this method. These and analyses of CT sections and natural fracture data (2) indicate that *Ar. ramidus* enamel is considerably thinner than that of *Australopithecus* but not as thin as in *Pan* [as originally reported in (1)] (Fig. 4 and figs. S31 and S32).

Of particular importance is that *Ar. ramidus* molars do not exhibit enamel distribution patterns characteristic of *P. troglodytes* and *P. paniscus*. Both *Pan* species have similar crown structure and enamel distribution patterns (Fig. 4), although *P. paniscus* molars exhibit a higher cuspal topography, perhaps related to greater reliance on fibrous food (46, 47). *Ar. ramidus* lacks the thin occlusal fovea enamel of *Pan* and in this regard is similar to both *Australopithecus* and Miocene forms such as *Dryopithecus* (Fig. 4). The *Pan* condition is most likely derived, probably associated with an increased reliance on higher-canopy ripe fruit feeding.

Despite the generalized molar structure common to both *Ar. ramidus* and *Australopithecus*, the adaptive difference between the two is expressed by enamel tissue volume, which we consider to broadly track net resistance to abrasion. Modern ape species exhibit a near-isometric relation between molar durability (measured as volume of enamel tissue available for wear per unit occlusal area) and tooth size, despite diverse dietary preferences and crown anatomy (Fig. 4). *Ar. ramidus* falls near this isometric continuum, but *Australopithecus* does not. *Australopithecus* molars achieve greater functional durability from increased enamel volume. *Au. boisei* occupies an extreme position distant from the modern ape baseline. Thus, both tooth size and enamel thickness and volume suggest a substantial adaptive shift from *Ardipithecus* to *Australopithecus*.

This is further expressed in molar macro- and microscopic wear patterns. In contrast to *Australopithecus*, *Ar. ramidus* molars did not wear flat but instead retained stronger buccolingual wear slopes. The Aramis *Ar. ramidus* dentition also exhibits consistently weak M1 to M3 wear gradients (48). Microwear of the *Ar. ramidus* molars tends to differ from that of *Au. afarensis*, the latter characterized by a dominance of buccolingually oriented scratches (49). In contrast, the *Ar. ramidus* molars tend to exhibit finer and more randomly oriented striae (fig. S33). Collectively, the wear evidence suggests that *Ar. ramidus* consumed a less abrasive diet and engaged in less masticatory grinding than *Australopithecus*.

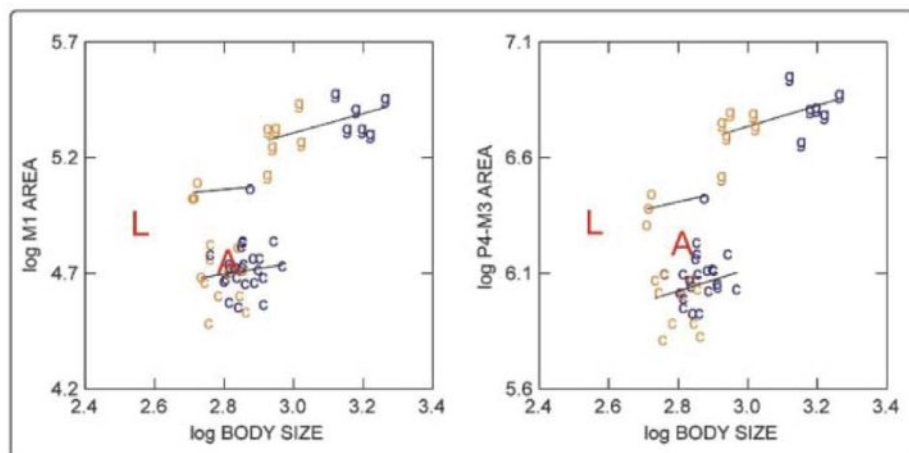


Fig. 3. Relative postcanine dental size in *Ar. ramidus*. Postcanine size is compared directly in reference to associated postcranial elements; x axis is natural log of the size variable (body size proxy) of Lovejoy *et al.* (23), derived from four metrics of the talus and five metrics of the capitate; y axis is natural log of the square root of the sum of calculated areas (mesiodistal length multiplied by buccolingual breadth) of lower M₁ (left) and lower P₄ to M₃ (right). A, *Ar. ramidus* ARA-VP-6/500; L, *Au. africanus/afarensis* A.L. 288-1; C, *Pan troglodytes troglodytes*; G, *Gorilla gorilla gorilla*; O, *Pongo pygmaeus* (males blue, females red).

Enamel thickness and phylogenetic implications. Since the initial description of *Ar. ramidus* as a new species of Hominidae (1), its relatively thin molar enamel has been a focus of attention. Some authors have suggested that its thin enamel might be a shared derived feature with *Pan* (7). The fuller study of molar enamel thickness and patterns outlined above establishes the

following: (i) Although *Ar. ramidus* enamel is thinner than that of *Australopithecus*, it is not as thin as *Pan*'s; (ii) the thin enamel of *Pan* molars can be considered a part of a structural adaptation to ripe fruit frugivory (46) and therefore differs from the *Ar. ramidus* condition. Furthermore, the distinct internal structure of *Pan* molars seems lacking in *Ar. kadabba*, *O. tugenensis*, and *S.*

tchadensis (4, 8, 10). Hence, the *Pan* condition is best considered derived relative to the ancestral and early hominid conditions.

Conclusions. Multiple lines of morphological evidence suggest that *Ar. ramidus* was a generalized omnivore and frugivore that did not rely heavily on either ripe fruits (as in *Pan* or *Pongo*), fibrous plant foods (as in *Gorilla*), or hard and

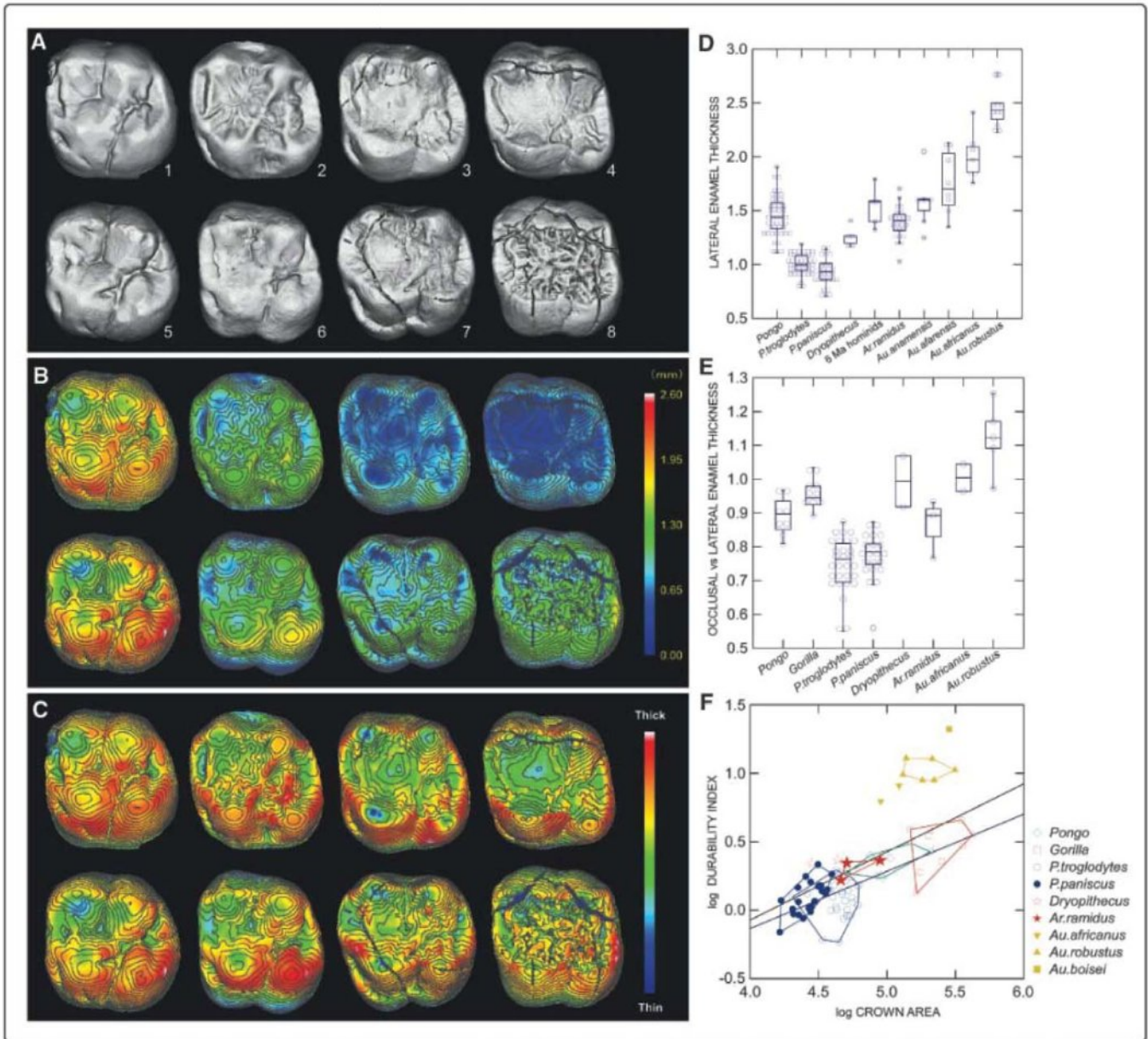


Fig. 4. Enamel thickness and distribution patterns in *Ar. ramidus*. Left panels: micro-CT-based visualizations of maxillary first molars in arbitrary size. (A) Outer enamel surface; (B) enamel thickness in absolute thickness scale superimposed on topographic contours; (C) enamel thickness in relative scale to facilitate comparison of pattern. The molars [labeled in (A)] are as follows: 1 and 5, *Au. africanus* Sts 24 (mirrored) and Sts 57; 2, *Dryopithecus branchoi*; 6, *Ar. ramidus* ARA-VP-1/3288; 3, *Pan troglodytes*; 4, *Pan paniscus*; 7, *Gorilla gorilla*; 8, *Pongo pygmaeus*. The *Pan* species share a broad occlusal basin and thin occlusal enamel. Both *Ar. ramidus* and *D. branchoi* are thinner-enamelled than *Australopithecus* but share with *Australopithecus* a generalized distribution pattern. (D) Maximum lateral enamel thickness, showing that *Ar. ramidus* enamel is thicker than those of

Pan and *D. branchoi* and thinner than that of *Australopithecus* species. Horizontal line is median; box margins are central 50% range. (E) Ratio of occlusal (volume/surface area) to lateral (average linear) enamel thicknesses, showing that *Pan* is unique in its distinctly thin occlusal enamel. (F) Molar durability (enamel volume per unit occlusal view crown area) plotted against projected occlusal view crown area. An isometric line (slope of 0.5) is fitted through the centroid of the three measured *Ar. ramidus* molars. The least squares regression ($y = 0.418x - 1.806$) of the combined modern ape sample is also shown. This slope does not differ significantly from isometry. *Ar. ramidus* and *D. branchoi* are close to, and *Australopithecus* species considerably above, the regression line, indicating greater enamel volume available for wear in *Australopithecus* molars. See (2) for further details.

tough food items (as in *Pongo* or *Australopithecus*). *Ar. ramidus* also lacked adaptations to abrasive feeding environments (unlike *Australopithecus*). These inferences are corroborated by the isotopic analysis of enamel, which indicates that *Ar. ramidus* predominantly consumed (~85 to 90%) C_3 plant sources in woodland habitats and small patches of forest (50), thus differing from both savanna woodland-dwelling chimpanzees (>90% C_3) and *Australopithecus* spp. (>30% C_4) (51).

Conversely, extant *Pan* and *Gorilla*, each with its distinctive dental morphology, are best considered derived in their dietary and dental adaptations. This is consistent with the *Ar. ramidus* postcranial evidence and its interpretations (11, 23) and strengthens the hypothesis that dental and locomotor specializations evolved independently in each extant great ape genus. This implies that considerable adaptive novelty was necessary to escape extinction in the Late Miocene forest and woodland environments.

These analyses also inform the social behavior of *Ar. ramidus* and its ancestors. The dental evidence leads to the hypothesis that the last common ancestors of African apes and hominids were characterized by relatively low levels of canine, postcanine, and body size dimorphism. These were probably the anatomical correlates of comparatively weak amounts of male-male competition, perhaps associated with male philopatry and a tendency for male-female codominance as seen in *P. paniscus* and ateline species (52, 53).

From this ancestral condition, we hypothesize that the *P. troglodytes* lineage secondarily enhanced its canine weaponry in both sexes, whereas a general size reduction of the dentition and cranium (25) occurred in the *P. paniscus* lineage. This suggests that the excessively aggressive intermale and intergroup behavior seen in modern *P. troglodytes* is unique to that lineage and that this derived condition compromises the living chimpanzee as a behavioral model for the ancestral hominid condition. The same may be the case with *Gorilla*, whose social system may be a part of an adaptation involving large body size, a specialized diet, and marked sexual dimorphism.

In the hominid precursors of *Ar. ramidus*, the predominant and cardinal evolutionary innovations of the dentition were reduction of male canine size and minimization of its visual prominence. The *Ar. ramidus* dental evidence suggests that this occurred as a consequence of selection for a less projecting and threatening male upper canine. The fossils now available suggest that male canine reduction was well underway by 6 million years ago and continued into the Pliocene. Further fossils will illuminate the tempo and mode of evolution before 6 million years ago.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/69/DC1

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4 May 2009; accepted 18 August 2009

10.1126/science.1175824

The Great Divides: *Ardipithecus ramidus* Reveals the Postcrania of Our Last Common Ancestors with African Apes

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Genomic comparisons have established the chimpanzee and bonobo as our closest living relatives. However, the intricacies of gene regulation and expression caution against the use of these extant apes in deducing the anatomical structure of the last common ancestor that we shared with them. Evidence for this structure must therefore be sought from the fossil record. Until now, that record has provided few relevant data because available fossils were too recent or too incomplete. Evidence from *Ardipithecus ramidus* now suggests that the last common ancestor lacked the hand, foot, pelvic, vertebral, and limb structures and proportions specialized for suspension, vertical climbing, and knuckle-walking among extant African apes. If this hypothesis is correct, each extant African ape genus must have independently acquired these specializations from more generalized ancestors who still practiced careful arboreal climbing and bridging. African apes and hominids acquired advanced orthograde in parallel. Hominoid spinal invagination is an embryogenetic mechanism that reoriented the shoulder girdle more laterally. It was unaccompanied by substantial lumbar spine abbreviation, an adaptation restricted to vertical climbing and/or suspension. The specialized locomotor anatomies and behaviors of chimpanzees and gorillas therefore constitute poor models for the origin and evolution of human bipedality.

Thomas Huxley published *Evidence as to Man's Place in Nature* (1) only 4 years after Darwin's *On the Origin of Species*. Its frontispiece featured a human skeleton and four suspensory adapted apes, each posed upright and each obviously more human-like than any pronograde Old World monkey. By century's end, Keith was enumerating a cornucopia of characters in support of a brachiationist human past (2). Even our pericardial-diaphragmatic fusion, hepatic bare area, and colic mesenteries were interpreted as adaptations to orthograde, evolved to tame a flailing gut in the arboreal canopy. Bipedality was simply habitual suspension brought to Earth (3). The "suspensory paradigm" for early hominid evolution was born.

Challenges, however, were mounted. Straus enumerated disconcertingly primitive human features in "The Riddle of Man's Ancestry" (4), and Schultz doubted that brachiation "... opened the way automatically for the erect posture of modern man" [(5), pp. 356–357]. Although withdrawal of the ulna from its primitive pisotriquetral recess was thought to be the sine qua non of suspension (6), a functional equivalent was dis-

covered to have evolved in parallel in the wrists of never-suspensory lorises (7). African ape knuckle-walking (8), considered by many too bizarre to have evolved independently in *Gorilla* and *Pan*, came to be viewed in light of emergent molecular phylogenetics (9) as a natural successor of suspensory locomotion—and by some as the almost-certain default engine of bipedality (10).

A flood of morphometric analyses appeared to confirm arguments for knuckle-walking hominid ancestors [reviewed in (11)], even though hints of the behavior were also seen in captive orangutans (12). Knuckle-walking was surmised to be a natural consequence of irreversible modifications of the forelimb skeleton to facilitate advanced suspension and vertical climbing (11). It was thereby hypothesized to be an adaptive signal of the first two phases of a deterministic succession leading to bipedality: advanced suspension/vertical climbing → terrestriality/knuckle-walking → bipedality.

A compendium of observations of chimpanzees and bonobos performing upright stance and locomotion followed. Accumulating molecular biology propelled this troglodytian paradigm (conceived as a natural succession to its older, suspensory counterpart) to near-consensus. Chimpanzee-human protein homologies and DNA base sequence comparisons (9, 13–16) established *Homo* and *Pan* as likely sister clades [today further confirmed by comparative genomics (17, 18)]. The only question remaining seemed to be whether the bonobo or chimpanzee represented the best living proxy for the last common ancestor (19–22).

The Chimpanzee model and *Australopithecus*.

The discovery and recognition of the then-primitive *Australopithecus afarensis* during the 1970s (23) pushed the hominid record back to 3.7 million years ago (Ma). Although its postcranium was recognized to harbor unusually sophisticated adaptations to bipedality [reviewed in (24)], a feature confirmed by human-like footprints at Laetoli (25, 26), many interpreted these fossils to represent the closing argument for the troglodytian paradigm [see, e.g., (27)]. Only the recovery of earlier, chimpanzee-like fossils from the Late Miocene seemed necessary to complete this scenario [even though newer *Australopithecus* fossils have led at least one discoverer to doubt a chimpanzee-like ancestry (28)]. Until now, the few available fossils of appropriate antiquity have remained largely uninformative (29–31).

The *Ardipithecus ramidus* fossils from 4.4 Ma Ethiopia are obviously not old enough to represent the chimpanzee/human last common ancestor (CLCA; the older common ancestor of hominids and both *Gorilla* and *Pan* is hereafter the GLCA). However, their morphology differs substantially from that of *Australopithecus*. The *Ar. ramidus* fossils therefore provide novel insights into the anatomical structure of our elusive common ancestors with the African apes. For that reason, and because of its phylogenetic position as the sister taxon of later hominids (32), this species now provides opportunities to examine both the suspensory and troglodytian paradigms with greater clarity than has previously been possible. Here we first provide evidence of limb proportions, long considered to bear directly on such issues, and then review key aspects of the entire *Ar. ramidus* postcranium. Comparing the basic proportions and postcranial anatomy of *Ar. ramidus* (Fig. 1) with those of apes enables us to propose the most probable anatomies of the last common ancestors of *Gorilla*, *Pan*, and the earliest hominids. Much of the relevant information on *Ar. ramidus* is based on the partial skeleton from Aramis (32).

Body mass. The geometric means of several metrics of the capitate and talus are strongly related to body mass in extant primates (correlation coefficient $r = 0.97$; fig. S1), and can be used to estimate body mass in *ARA-VP-6/500*, as well as in *A.L. 288-1*. Restricting the sample to large-bodied female hominoids predicts that *ARA-VP-6/500* had a mass of about 51 kg. The metrics for *A.L. 288-1* fall below those of all extant hominoids. We therefore used the female anthropoid regression to estimate the body mass of *A.L. 288-1* (26 kg), which is consistent with previous estimates (33) (table S1). Based on several shared metrics, *ARA-VP-7/2*, a partial forelimb skeleton (32), was slightly smaller than *ARA-VP-6/500* [supporting online material (SOM) Text S1].

Given the apparent minimum body size dimorphism of *Ar. ramidus* (32, 34), the predicted

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body mass of *ARA-VP-6/500* serves as a reasonable estimate for the general body mass of *Ar. ramidus*. Although *ARA-VP-6/500* was one of the larger individuals of the Aramis sample (32), it was probably more representative of its species than was *A.L. 288-1* [the latter clearly lies at the lower end of the *Au. afarensis* species range based on larger samples (35)]. Unfortunately, *ARA-VP-6/500* tells us little about the body mass of the CLCA and GLCA because these predate *Ar. ramidus* by wide margins and may have still been primarily arboreal. The limited available (mostly dental and cranio-mandibular) samples indicate that the size of Late Miocene hominids (29–31) was similar to that of *Ar.*

ramidus (34), and estimated body weight for the 6 Ma *Orrorin* femoral remains is 30 to 50 kg (36). Although body mass in early Miocene forms appears to have varied greatly (37, 38), it is likely that the CLCA and GLCA were either equal to or smaller than *Ar. ramidus*, and possibly even substantially so. Only additional fossils can resolve this issue.

Limb segment proportions. Radial, ulnar, and tibial lengths can be accurately determined for *ARA-VP-6/500* (SOM Text S1). The specimen's radius/tibia ratio (0.95; fig. S2) is similar to those of generalized above-branch quadrupeds such as the Old World monkey *Macaca* (0.90 to 0.94; table S2) and the Mio-

cene ape *Proconsul heseloni* (0.88 in *KNM-RU 2036*) (38). The ratio is unlike that of African apes (*P. troglodytes*, 1.11 ± 0.04 ; *Gorilla*, 1.13 ± 0.02) (39) and is, remarkably, 17 standard deviations below that of *Pongo* (1.47 ± 0.03).

The *Ardipithecus* skeleton's nearly intact tibia allows estimation of femoral length because the crural index (CI: tibia length/femur length $\times 100$) is highly conserved in African apes and humans (5, 40) (81 to 84; SOM Text S1). Tibial length in *A.L. 288-1* can likewise be estimated from its effectively complete femur. Although no humerus was recovered for *ARA-VP-6/500*, one belonging to *ARA-VP-7/2* is almost complete and can be used to estimate humerus length in *ARA-VP-6/500* by simple proportion of shared elements (SOM Text S1). The *A.L. 288-1* humerus is intact, and its radius length was previously estimated by regression (41). These data allow calculation of the more familiar intermembral index (IMI; forelimb length/hindlimb length $\times 100$). The IMIs of both specimens resemble those of *Proconsul* and Old World monkeys (table S3).

ARA-VP-6/500 also allows interpolation of other key limb proportions. The brachial indices (BI: radius length/humerus length $\times 100$) of *Proconsul*, *Equatorius*, *A.L. 288-1*, and *ARA-VP-6/500* are each within the observed range of *Pan* (fig. S3). It is therefore likely that the BI has remained largely unmodified since the GLCA, especially in light of the relationship of radius length to estimated body mass (fig. S4). In contrast, the BIs of *Homo* and *Gorilla* are both derived, albeit by obviously different routes (fig. S3). Humans have greatly shortened radii in conjunction with their novel antebrachial/manual proportions for grasping and manipulation [(41, 42) and see below]; *Gorilla* appears to have experienced both humeral elongation and possibly slight radial shortening (figs. S4 and S5), most likely to reduce joint stresses at the elbow imposed by the immense mass of adult males. The BIs of *Pan* and *Ar. ramidus* are similar (fig. S3), but *Pan* exhibits a much higher IMI (table S3). Therefore, both *Pan* and *Gorilla* have undergone forelimb elongation and hindlimb reduction since the GLCA (table S2 and figs. S4 to S6). The IMIs of hominids appear to have remained primitive until 2.5 Ma (41, 43). The relatively high BI of *Pongo* reflects its entirely different evolutionary history.

Manual anatomy and proportions. Compared to estimated body size, the manual phalanges of *Ar. ramidus* and *Gorilla* are long relative to those of the Miocene ape *Proconsul* (fig. S7). They are relatively even more elongate in *Pan*, but dramatically abbreviated in *Homo*. These conclusions are supported by similar calculations using the means of observed body mass (table S3). There is no evidence that the manual phalanges of *Au. afarensis* were elongated relative to those of *Ar. ramidus*.

In contrast to their manual phalanges, the posterior (medial) metacarpals 2 to 5 (Mc2-5) of *Proconsul* and *ARA-VP-6/500* are substantially

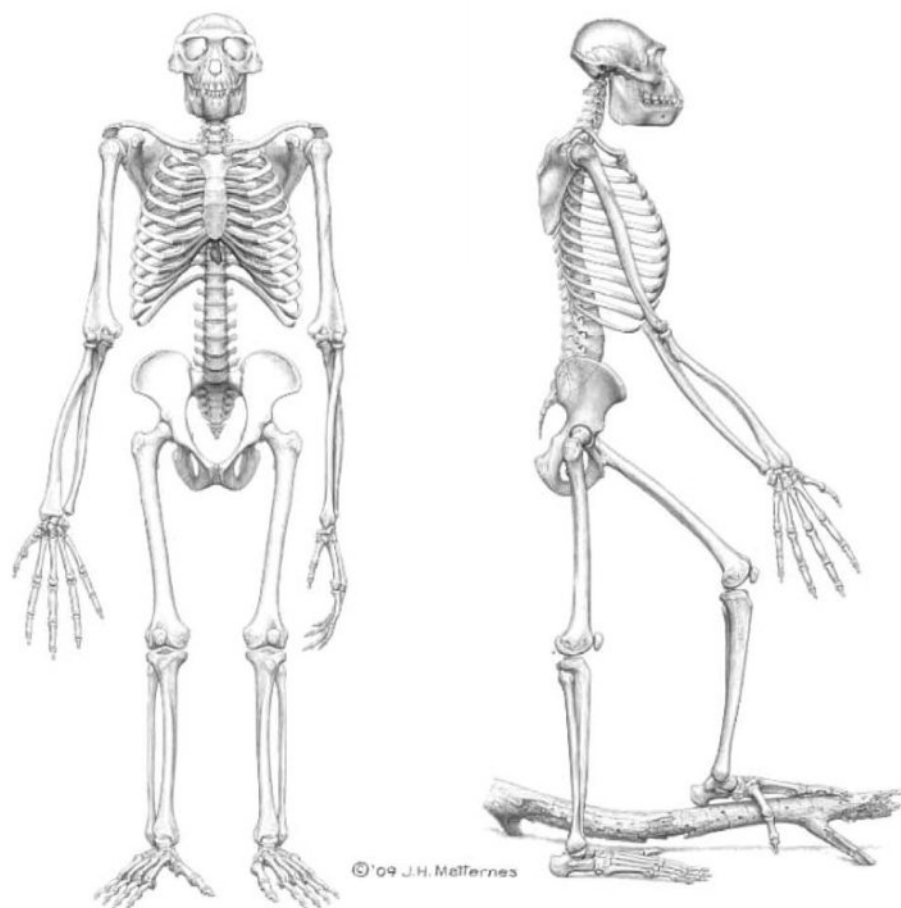


Fig. 1. Reconstructed frontal and lateral views of the skeleton of *ARA-VP-6/500*. Major long-bone lengths were determined directly from preserved skeletal elements (radius, tibia), by regression from adjacent elements (ulna), or by ratio and regression (humerus) from a marginally smaller forelimb skeleton (*ARA-VP-7/2*) via ratios of commonly preserved elements (SOM Text S1). All manual and pedal elements were drawn directly from casts. Pelvis was traced from frontal and lateral computer tomography (CT) scans of reconstructed pelvis (59). Vertebral column and thorax were based on six lumbar, 12 thoracics, and four sacra (58). No attempt has been made to indicate failure of lateral fusion between the transverse processes of S4 and S5 [i.e., failure of complete closure of either of the fourth sacral foramina (the state preserved in both *A.L. 288-1* and *KNM-WT 15000*)]. Such four-segment sacra may have been modal in *Ar. ramidus*, but the five-segment form shown here was also a likely variant of high frequency [for discussion, see (59)]. Pectoral girdle and thorax were based on preserved portions of clavicle, first rib, and common elements known in *Au. afarensis*. Skull and mandible were based on models generated by restoration of cranium using both CT/rapid prototyping and “cast-element-assembly” methods (79). Reconstruction by J. H. Matternes was based on full-scale (life-size) architectural drawings circulated among authors for multiple inspections and comments. Stature (bipedal) is estimated at 117 to 124 cm and body weight at 51 kg. [Illustrations: Copyright 2009, J. H. Matternes]

shorter than are those of any extant ape (figs. S8 and S9). Viewed in the context of relative limb length patterns (see above), as well as the anatomical details of the hand (44), the short Mcs of *Ar. ramidus* strongly suggest that *Pan* and *Gorilla* independently acquired elongate Mcs as a part of an adaptation to vertical climbing and suspensory locomotion. Elongation of Mc2-5 in African apes demanded heightened resistance to torsion and consequent fixation of the carpo-metacarpal joints within the central joint complex (CJC) (44).

The retention of the primitively short Mcs in *Ar. ramidus* suggests that the GLCA/CLCA also did not have elongate Mcs, and engaged in a form of above-branch quadrupedal locomotion involving deliberate bridging and careful climbing. We hypothesize that this was retained from Middle Miocene precursors of the GLCA. A retained short metacarpus would optimize palmar conformity to substrates, an adaptation later abandoned by extant African apes.

The thumb metacarpal of *ARA-VP-6/500* was more aptly proportioned for manual grasping than are those of extant apes (figs. S10 and S11) (44). In extant apes, elongation of the posterior (medial) metacarpus may have been achieved by increased expression of *Hoxd11* or one of its targets, which do not affect the first ray (SOM Text S2) (42, 45). However, the Mc1 of apes does seem moderately less robust than that of *Ar. ramidus*, and its soft tissues have undergone substantial involution (4, 42). This suggests that some degree of down-regulation of *Hoxd13* may have been responsible for elongation of the posterior (medial) metacarpus.

Ar. ramidus greatly illuminates the natural history of the thumb in higher primates. Its robusticity in hominids, while certainly enhanced during the past 3 million years, is nevertheless at least partially primitive. In contrast, in taxa adapted to vertical climbing and suspension, lengthening of the palm has become so dominant as to eclipse some of the thumb's function, a condition that has reached its apogee in *Ateles* and, to a lesser extent, large-bodied extant apes. These findings strongly suggest that the target of recently discovered major cis-regulatory modification of gene expression in the first ray (46) was not manual but pedal—it is the human hallux, not our largely primitive pollex, that is highly derived (47).

Additional relevant hand anatomy leads to the same conclusions. *Ar. ramidus* is the only hominid fossil thus far recovered with a metacarpal head reminiscent of the metacarpophalangeal (MP) joint structure seen in many Miocene hominoids [such as *Equatorius*, *Proconsul*, *Dryopithecus*, and *Pierolapithecus* (48)]. The collateral ligament facets in these taxa collocate with deep symmetric invaginations of the metacarpal head's dorsum. This morphology is typical of Old World monkeys and is thereby associable with substantial dorsiflexion of the MP joint, an obvious manifestation of their palmigrady. The

trait is only moderately expressed in *Oreopithecus*. Modern human and orangutan MP joints are substantially less constricted, and neither taxon exhibits appreciable locomotor-related MP dorsiflexion.

Constricted metacarpal head morphology appears to be primitive because it is still partially present in *Ar. ramidus*, albeit substantially reduced compared to early Miocene hominoids and Old World monkeys. Its retention suggests moderately frequent MP dorsiflexion, a finding consistent with the remarkable adaptations to palmigrady seen in the *Ar. ramidus* wrist [see below and (44)].

The metacarpal heads of knuckle-walking apes are also somewhat constricted by their collateral facets, but are heavily flattened and broadened to withstand excessive compression during dorsiflexion. Constriction by their collateral ligament facets is therefore only minimal. Moreover, the origins of their collateral ligaments have been substantially expanded volarly, presumably because such positioning improves their capacity to restrict abduction or adduction during MP dorsiflexion imposed by knuckle-walking. Joint flattening enhances cartilage contact and is likely at least partially a cartilage-modeling trait [cartilage modeling; Type 4 (49)].

Loss of MP dorsiflexion in *Pongo* is readily explicable by its extreme metacarpal and phalangeal elongation and curvature. These can safely be presumed to have eliminated any appreciable functional MP dorsiflexion. Modern humans lack any dorsiflexion because our hand plays no important role in locomotion. The trait is also absent in *Au. afarensis*, suggesting that either its hand no longer played any role in locomotion, or that such use no longer included an MP dorsiflexive component of palmigrady. The former seems far more likely, given the paramount adaptations to bipedality in the species' lower limb (24, 50, 51).

The primitive metacarpal head morphology within the overall primitive hand anatomy (44) of *Ar. ramidus* carries obvious implications for reconstruction of GLCA/CLCA locomotion. The unique combination of marked midcarpal mobility, ulnar withdrawal, and moderate MP dorsiflexion in *Ar. ramidus*, probably mostly primitive retentions, implies that the GLCA/CLCA locomotor pattern was also characterized by some form of arboreal palmigrade quadrupedality, unlike that in any extant descendant great ape.

Finally, it is clear now that phalangeal length of *Ar. ramidus* is not related to suspensory locomotion, but instead reflects a more general grasping adaptation. This renders phalangeal length moot regarding the hypothesis that manual (or even pedal) phalangeal lengths are an active signal of suspensory locomotion in *Au. afarensis* [contra (52, 53)]. It is more probable that selection had not reduced their length in the younger species, and that such reduction did not occur until selection for tool-making became more intense later in the Pliocene (43, 54).

Pedal proportions. Pedal phalangeal evolution appears to have closely paralleled its manual

counterpart in each clade (compare figs. S7 and S12). However, pedal phalanges of African apes and hominids appear to have been substantially abbreviated, rather than elongated. Functional demands of terrestrial locomotion, perhaps similar to those acting on papionins (which also exhibit pedal phalangeal shortening), are a probable explanation. *Pongo* represents a marked contrast, with substantial pedal phalangeal elongation. It is thus reasonable to infer that the GLCA/CLCA's pedal phalanges were longer than those of the partially terrestrial extant African apes and *Ar. ramidus*.

The metatarsus of *Ar. ramidus*, chimpanzees, and gorillas presents a striking contrast to their metacarpus. Like the foot phalanges, the metatarsals also appear to have been universally shortened in all hominoids subsequent to *Proconsul* (figs. S13 and S14) (47). The basis of this universal shortening, however, is somewhat unclear, because tarsal evolution contrasts dramatically in hominids and African apes. The modern ape foot has obviously experienced functional reorganization into a more hand-like grasping organ. The *Ar. ramidus* foot did not. This suggests that substantial elements of a more lever-based, propulsive structure seen in taxa such as *Proconsul* and Old World Monkeys [robust plantar aponeurosis; retained quadratus plantae; robust peroneal complex (47)] were preserved in the GLCA/CLCA. These structures were sacrificed in both African ape clades to enhance pedal grasping for vertical climbing (55, 56). The moderate shortening of the metatarsus in *Ar. ramidus* and both African apes may therefore simply reflect negative allometry of metatarsal (Mt) lengths with an increase in body size. The human foot has been lengthened primarily by tarsal elongation (5, 47), presumably because of the likely high failure rate of metatarsal shafts during forceful fulcrumation.

In summary, a comparison of the pedal proportions of *Ar. ramidus* and the extant African apes suggests that the GLCA/CLCA hindlimb remained dominant for body mass support during bridging and arboreal clambering, to the extent that it later proved permissive to bipedality in transitionally terrestrial hominids.

Trunk structure. Knowledge of the role of selector genes in early vertebral column formation [especially the role of the *Hox* code on column differentiation (57, 58)] has advanced our ability to interpret the vertebral formulae of extant hominoids. It now appears that the modal number of lumbar vertebrae in *Australopithecus* was six, and that a four-segment sacrum was also probably common (57, 58). This axial formula is unlike that of any extant ape. Comparison of the axial columns of extant species further indicates that postoccipital somite number in the GLCA/CLCA was probably either 33 or 34, and that lumbar column reduction occurred independently in chimpanzees, bonobos, gorillas, and hominids. This probably resulted from either transformation of vertebral identities,

or a combination of such transformation and reduction in the number of somites contributing to the lumbosacral region (fig. S15). The most likely vertebral patterns for *Ar. ramidus* are therefore those also inferred for the GLCA/CLCA and *Australopithecus*.

Pelvic structure indicates that *Ar. ramidus* retained a primitive spine. Its iliac and acetabular regions establish not only that it was habitually bipedal when terrestrial, but also that this was achieved by combining situational anterior pelvic tilt to accentuate substantial lordosis during upright walking (59). Such rotation placed the still partially primitive anterior gluteal musculature into a position of functional abduction for single support stabilization. In contrast to *Ar. ramidus*, *Au. afarensis* is known to have exhibited highly evolved mechanisms of hip abduction, confirmed by the distinctly stereotypic trabecular profile of its femoral neck (24).

The *Ar. ramidus* pelvis retained other elements in common with extant African apes (and presumably the GLCA/CLCA). These include a long, expansive and rugose ischial region and shorter pubic rami (but not a long pubic corpus) (59). The species' highly flexible lower lumbar column, coupled with its narrower interacetabular distance, still must have provided a moderately reflexive hindlimb for arboreal climbing. Not until hominids became habitually terrestrial bipeds with broad interacetabular distances, reduced and angulated ischial tuberosities (possibly indicating hamstring deceleration of the hindlimb at heel strike), and extremely shortened, flared, and broadened ilia did they then exchange such flexibility for the much more rigid constraints of lower-limb stabilization that characterize *Australopithecus* (50, 51).

The combined pelvic and vertebral data imply that the morphological elements of extant great apes emerged separately rather than in concert. Vertebral column invagination and its associated gracilization of the retroauricular pelvic space preceded specialized iliac modification and the radical lumbar column shortening seen in the African apes (58). The *ARA-VP-6/500* pelvis shows that hominid ilia shortened and broadened to establish permanent lumbar lordosis. African ape ilia were instead modified to increase abdominal stiffness. The posterior pelvic changes and pronounced lordosis in hominids subsequently promoted even more dramatic vertebral column invagination (60). This trend is eventually reflected in more dorsally oriented transverse processes of hominid thoracic vertebrae compared to those of apes (60). In extant apes, vertebral column invagination and shortening were acquired both independently and non-contemporaneously, the first being a deeply rooted embryogenetic mechanism that posterolateralized the pectoral girdle for a more lateral-facing glenoid; the second, an independent means of increasing abdominal rigidity. We hypothesize that hominids never participated in the second (SOM Text S3), having rather evolved

from a careful climber in which deliberate bridging placed no undue stress on the lower spine. Not until the ancestors of African apes embarked (separately) on their adaptations to vertical climbing and suspension did the lumbar spine undergo its dramatic reduction in length.

The last common ancestors. Integration of the data and observations reviewed above allows us to hypothesize about the postcranial adaptations and locomotion of the GLCA and CLCA. The extensive array of highly distinctive specializations seen in modern *Gorilla* and *Pan* (in part shared with *Pongo*) indicates that these are derived features most likely related to vertical climbing and suspension.

Not only does *Ar. ramidus* fail to exhibit these specialized modifications, it exhibits others (e.g., a palmar position of the capitate head that facilitates extreme dorsiflexion of the midcarpal joint rather than its limitation; a robust os peroneum complex limiting plantar conformity to substrates rather than its facilitation) that are effectively their functional opposites. The expression of some of these characters (e.g., capitate head position) is even more extreme than it is in either the Miocene apes preceding *Ardipithecus* or in *Australopithecus* that follows. It is therefore highly unlikely that *Ar. ramidus* descended from a *Pan/Gorilla*-like ancestor and then (re)evolved such extreme characters. Conversely, some other detailed differences in *Pan* and *Gorilla* structure [e.g., scapular form (61), iliac immobilization of lumbar vertebrae (58), appearance of a prepollex (62)] suggest that each of these ape clades independently acquired their anatomical adaptations to vertical climbing and/or suspension.

Therefore, we hypothesize that *Ar. ramidus* retains much of the ancestral GLCA and CLCA character states, i.e., those that relate to above-branch quadrupedality. In particular, contra *Gorilla* and *Pan*, the GLCA carpometacarpal, midcarpal, radiocarpal, and ulnotrochlear joints must have lacked notable adaptations to suspension and/or vertical climbing (44). The GLCA foot seems to have been only partially modified for manual-like grasping. Its hindlimb remained fully propulsive at its midtarsal and tarsometatarsal joints (47). Although its shoulder joint must have been fully lateralized, its lumbar column nevertheless was still long (58) (fig. S15). Its limb proportions were still primitive (see earlier). If body size was as large as in *Ar. ramidus*, it may have been too large for habitual, unrestricted above-branch quadrupedality, but this remains uncertain. Assuming considerable reliance on arboreal subsistence, it is likely that body mass did not exceed 35 to 60 kg [i.e., combined probable range of *Ar. ramidus* and 6 Ma *Orrorin* (36)].

The GLCA picture that emerges, therefore, is one of generalized, deliberate bridging with quadrupedal palmigrady and preference for large-diameter substrates. This may have involved either suspension or vertical climbing, but without sufficient frequency to elicit morphological

adaptations specific to these behaviors. It is likely that these hominoids ranged mostly in the lower canopy, and perhaps were even partially terrestrial. However, their mode of terrestrial locomotion remains unknown.

The GLCA therefore represents a foundation for two adaptive paths. *Gorilla* and *Pan* independently specialized for both suspension and vertical climbing (and eventually knuckle-walking). Gorillas might have acquired larger body size in relation to mixing higher-canopy frugivory with a more terrestrial herbaceous or folivorous dietary component. Lacking definitive fossil evidence, it is currently impossible to determine when the large body mass of *Gorilla* evolved, but it probably occurred in concert with its more herbaceous diet. The 10 Ma *Chororapithecus*, which shows incipient signs of *Gorilla*-like molar morphology (63), may be an early representative of the *Gorilla* clade. If so, then this clade's shift toward increased body mass and terrestriality must have occurred early in its phyletic history.

The other adaptive pathway retained palmar flexibility, with a short metacarpus that lacked notable syndesmotric restriction. This was combined with retention of an essentially rigid midtarsal joint that was insufficiently flexible to perform vertical climbing (55, 56), but was fully satisfactory for less specialized careful climbing, clambering, and bridging. This is the hypothesized structure of the CLCA, from which *Pan* would have evolved a greater reliance on vertical climbing and suspension than occurred in the *Gorilla* clade, never reaching as large a body size.

In contrast to *Pan*, the forebears of *Ar. ramidus* early in the hominid clade must have relied increasingly on lower arboreal resources and terrestrial zones, without being dependent on higher-canopy resources (such as ripe fruits). From the comparative evidence now available from *Ar. ramidus* and *Pan* dental anatomy and isotopes, we posit that the chimpanzee clade increasingly developed a preference for (or dependency on) ripe fruit frugivory, whereas hominids retained a more primitive dental complex adequate for the range of transitional arboreal/terrestrial resources (34).

The likely K-selected demographic adaptation of all hominoids in a setting of almost certain competition with the surging Old World monkey radiation would have been a major factor (64, 65) driving such very different evolutionary trajectories of early African apes and hominids. The earliest fossil evidence for cercopithecoid radiation (an early colobine) is now close to 10 Ma (66). A much better record of both fossil hominoids and cercopithecoids from the late Middle to early Late Miocene is needed to clarify these suggested patterns of ape-cercopithecoid evolution.

Orthograde, suspension, knuckle-walking, and bipedality. *Ar. ramidus* affords new insights into ape and hominid bauplan evolution

(Fig. 2 and Table 1). The most fundamental is the clear demonstration that the GLCA lacked the suspensory adaptations long recognized to be common to all extant apes.

The chimpanzee and gorilla clades each independently increased their reliance on higher-canopy resources, and modified characters originally associable with advanced bridging to those more useful in vertical climbing and suspension. These include an elongated posterior (medial) metacarpus, broadened radiocarpal joint with reduced midcarpal mobility, syndesmotic and morphologically buttressed carpometacarpal joints, expanded long antebrachial flexor tendons, a redistributed long pollical flexor tendon (to the elongated second ray), a modified entheses for the deltopectoral complex, a retroflexed trochlear notch, elongate forelimbs (44), abbreviated hindlimbs, elimination of the os peroneum complex (47), lumbar column reduction (58), and iliac fixation of remaining lumbar [acquired by iliac elongation and sacral narrowing (58, 59)]. Viewed from the perspective of *Ar. ramidus*, all of these can now be visualized as having been acquired independently. All represent adaptations related directly to suspension, vertical climbing, and/or knuckle-walking.

In African apes, terrestrial travel may have become the primary means of overcoming expanding canopy gaps. A return to partial terrestrial pronogrady would have necessitated compensatory energy-absorptive mechanisms to ameliorate ground reaction in heavily modified forelimbs (which would have suffered an increased risk of injury). Knuckle-walking filled this role because it promotes eccentric contraction and/or energy dissipation (and storage) in the wrist and digital flexors (especially their connective tissue components) during impact loading in a completely extended forelimb, without compromising the animal's newly acquired adaptations to either suspension or vertical climbing (44). More elaborate mechanisms of negotiating gaps in trees (67) evolved separately in orangutans, in which both manual and pedal rays radically elongated, possibly to more effectively gather and assem-

ble multiple lianas necessary to negotiate such gaps.

Thus, *Ar. ramidus* allows us to infer that GLCA anatomy was exaptive for suspension and vertical climbing. Early hominids continued to practice palmigrade, above-branch quadrupedal clambering. Ulnar retraction, common to both *Pan* and *Gorilla*, therefore appears to have emerged for forelimb flexibility as part of arboreal clambering and bridging before the GLCA (7), and not as an adaptation to suspension [as has been argued (6)]. Initialized in forms like *Proconsul*, the combination of enhanced forelimb flexibility and hindlimb propulsive dominance, without anatomical modifications for forelimb suspension, may have reached an apogee in the GLCA.

These observations also conform to evidence available from the steadily increasing Miocene hominoid fossil record. European near-contemporaries of the African CLCA to GLCA exhibited only various degrees of adaptation to suspension, suggesting a separate Miocene trend toward increasing forelimb dominance. At 12 Ma, *Pierolapithecus* had ulnar withdrawal and partial spinal invagination (68), but likely retained a long lumbar spine. Its hand lacked the degree of metacarpal or phalangeal elongation seen in extant apes. More recent *Dryopithecus*, which did display both an African ape-like CJC (44) and elongate metacarpals relative to body size, nevertheless retained palmigrady (68, 69). Suspensory locomotion was therefore likely independently derived (minimally) in *Dryopithecus*, *Pan*, and *Gorilla* (and certainly so in *Pongo*). Hypotheses that hominid ancestry included suspensory locomotion and vertical climbing (52, 53), as projected from electromyographic and kinematic analyses of living ape behavior, are now highly unlikely.

From their beginning, accounts of human evolution relied on postural similarities between living humans and apes. The inference that habitual orthogrady was central to the origin of bipedality has been taken as largely self-evident (2, 70). Until now, no fossils of sufficient age

and anatomical representation have been available for seriously testing these presumptions. *Ar. ramidus* requires comprehensive revision of such entrenched, traditional canons. Its anatomy makes clear that advanced orthogrady evolved in parallel in hominids and apes, just as it has in an array of other primates, both living and extinct [including prosimians such as *Propithecus* and *Megaladapis*, some ceboids, gibbons, and a variety of Miocene hominoids, especially *Nacholapithecus* (71), and *Oreopithecus* (72)]. The long-held view that dorsal transposition of the lumbar transverse processes onto their pedicles implies orthogrady is now falsified, because *Ar. ramidus* establishes that such relocation is a direct correlate of ventral invagination of the entire spinal column within a context of above-branch quadrupedal palmigrady that established increased shoulder mobility for bridging and clambering (SOM Text S3).

In hominids, from an above-branch quadrupedal ancestry, advanced orthogrady was the independent consequence of terrestrial bipedality made possible by a mobile lumbar spine and largely primitive limbs. It is sobering to consider one profound implication—if emergent hominids had actually become as adapted to suspension or vertical climbing as are living apes, neither bipedality nor its social correlates would likely have evolved. It is therefore ironic that these locomotor modes have played so prominent a role in explanations of bipedality. In retrospect, it seems clear that they would instead have likely prevented it (SOM Text S3).

Conclusions. *Ar. ramidus* implies that African apes are adaptive cul-de-sacs rather than stages in human emergence. It also reveals an unanticipated and distinct locomotor bauplan for our last common ancestors with African apes, one based on careful climbing unpreserved in any extant form. Elaborate morphometric statistical procedures were the culmination of a 20th-century trend toward objectivity, in which metrics came to be regarded as more informative than careful comparative anatomy—a trend accompanied by too many presumptions and too few

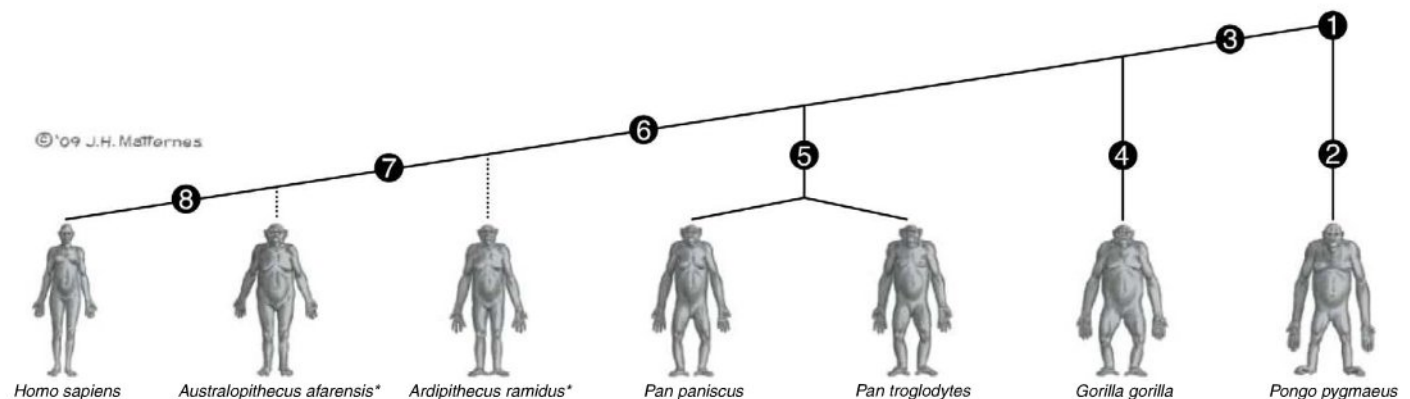


Fig. 2. Branching diagram to illustrate cladistic relationships of extant hominoids. Branching order among the extant forms shown here is well established by molecular evidence. The two fossil forms are possible phyletic ancestors of the human

clade, but are shown here in a sister relationship to the extant forms. Circled numbers indicate evolutionary derivations, itemized in Table 1, hypothesized to have occurred on each lineage. [Illustrations: Copyright 2009, J. H. Matternes]

Table 1. Evolutionary derivations of various hominoid clades with fossil and modern representation. Numbers refer to circles on Fig. 2.

1. Basal node. An inferred generalized ancestor of the great ape clade, which lived probably more than 18 Ma. We infer this primate to have been an above-branch palmigrade, plantigrade quadruped, with generalized limb proportions, an anteriorly oriented pectoral girdle, and long lumbar vertebral column with transverse processes located ventrally on their bodies. It would have also been characterized by an extensive postauricular iliac region for a massive erector spinae, a long olecranon process, an anteriorly oriented trochlea, a capitate head located mid-body, and a primitive central joint complex in the wrist. It would have featured a full wrist mortise with pisotriquetral contact and a moderately long midtarsus for fulcrumation on its metatarsal heads. It was presumably tailless (80).

2. Orangutan clade. Dramatic elongation of entire forelimb, posterior (medial) metacarpus and phalanges, extreme elongation of posterior (lateral) metatarsus and phalanges but abbreviation of thigh and leg, partial involution of first pedal and manual rays. Abbreviation of lumbar vertebral column (average four elements) by means of sacralization of lumbar vertebrae, reduction in axial length by two segments, and craniocaudal shortening of lumbar centra (58). Entrapment of caudal-most lumbar by articulation with variable cranial extension of ilia and reduction in breadth of sacral alae. Invagination of spine with posterolateralization of pectoral girdle and reduction of deltopectoral crest. Retroflexion of trochlear notch, extreme abbreviation of olecranon process, and elevation of lateral margin of trochlea. Ulnar withdrawal with elimination of wrist mortise. Modification of central joint complex for torsional resistance during suspension. Frequent postnatal fusion of os centrale and scaphoid.

3. Extant African ape and hominid clade (GLCA). Minor abbreviation of midtarsal length, elongation of manual phalanges, and shortening of posterior (lateral) metatarsus. Invagination of spine with posterolateralization of pectoral girdle, mediolateral proportionality shift of sacroiliac region, craniocaudally shortened vertebral centra, and relocation of lumbar transverse processes to corporopedicular junction or onto pedicle. Abbreviation of olecranon and elevation of lateral margin of trochlea. Ulnar withdrawal with elimination of wrist mortise (i.e., loss of pisotriquetral contact) and deepening of carpal tunnel. Fusion of os centrale to scaphoid.

4. Gorilla clade. Elongation of forelimb (by disproportionate elongation of humerus) and abbreviation of hindlimb (global change in limb proportions), moderate elongation of posterior (medial) metacarpus, moderate shortening of manual phalanges. Abbreviation of lumbar vertebral column (average 3.5 elements) by means of sacralization of lumbar and reduction in axial length by one segment (58). Entrapment of most caudal lumbar by articulation with cranially extended ilia and reduction in breadth of sacral alae. Moderate increase in cranial orientation of scapular spine and glenoid plane, reduction of deltopectoral crest. Retroflexion of ulnar trochlear notch with attendant abbreviation of olecranon process, expansion of long digital flexor (emergence of “flexion tubercle” on ulna), subduction or gracilization of long flexor tendon of thumb to expanded long digital flexor, increased osseo-ligamentous resistance to torque in CJC via distal prolongation of the volar portion of the capitate with corresponding evacuation of the Mc3 base (creating a mediolateral block-to-joint rotation by novel abutment of Mc2 and Mc3), dorsalization and enlargement of capitate head, frequent formation of prepollex (62) on trapezium, anterior relocation of collateral ligament attachments of metacarpophalangeal joints (with simultaneous expansion of attachment facets on metacarpals), expansion of metacarpal heads, reduced capacity for dorsiflexion at midcarpal joint. Introduction of lateral spiral pilaster with loss of third trochanter, elimination of os peroneal complex and substantial shortening of midtarsus, especially proximodistal abbreviation of navicular and cuboid, and abbreviation of dorsoplantar dimensions of metatarsal bases. Gracilization of plantar aponeurosis with loss of plantaris and reduction/elimination of quadratus plantae.

5. Basal chimpanzee/bonobo clade. Elongation of forelimb and abbreviation of hindlimb (global change in limb proportions) but less extreme than in 4. Substantial elongation of posterior (medial) metacarpus and further elongation of manual phalanges. Chimpanzees exhibit higher intermembral index than bonobos and are probably derived in this regard. Abbreviation of lumbar vertebral column (three or four elements) by transformation of vertebral type and/or reduction in axial length by one segment [chimpanzees and bonobos differ substantially in number of axial elements, and bonobo is clearly primitive in this regard (58)]. Entrapment of most caudal lumbar by articulation with cranially extended ilia and reduction in breadth of sacral alae. Further immobilization by novel lumbo-inguinal ligaments (81). Elongation of iliac isthmus. Dramatic mediolateral narrowing of scapula, marked increase in cranial orientation of scapular spine and glenoid plane, reduction of deltopectoral crest (intermuscular fusion?). Retroflexion of ulnar trochlear notch with attendant abbreviation of olecranon process, expansion of long digital flexor (emergence of “flexion tubercle” on ulna), subduction or gracilization of long flexor tendon of thumb to expanded long digital flexor, increased osseo-ligamentous resistance to torque in CJC via distal prolongation of the volar portion of the capitate with corresponding evacuation of the Mc3 base (creating a mediolateral block to joint rotation by novel abutment of Mc2 and Mc3), dorsalization and enlargement of capitate head, elimination of mobility in hamate/Mc4/Mc5 joint, possible gracilization of Mc1, reduced capacity for dorsiflexion at midcarpal joint, reduction and anterior relocation of collateral ligament “grooves” of metacarpophalangeal joints (but expansion of attachment facets on metacarpals), expansion of metacarpal heads. Introduction of lateral spiral pilaster with loss of third trochanter, elimination of os peroneal complex and substantial shortening of midtarsus, especially proximodistal abbreviation of navicular and cuboid, abbreviation of dorsoplantar dimensions of metatarsal bases. Gracilization of plantar aponeurosis with loss of plantaris and reduction/elimination of quadratus plantae.

6. Hominid clade, Late Miocene. Substantial superoinferior abbreviation of iliac isthmus and pubic symphyseal body, increased sagittal orientation and mediolateral broadening of ilium with novel growth plate for anterior inferior iliac spine, introduction of slight (obtuse) greater sciatic notch, (inferred) facultative lumbar lordosis, probable broadening of sacral alae to free most caudal lumbar for lordosis. Possible increased size and robusticity of fibularis longus, increased robusticity of second metatarsal base/shaft and doming of dorsal metatarsal heads related to toe-off.

7. Hominid clade, Mid-Pliocene. Shortening of ischial length and angulation of ischial tuberosity, further mediolateral expansion of iliac fossa with introduction of substantial (acute) greater sciatic notch, further invagination of lumbar vertebral column and fixation of lordosis (no longer facultative). Reduction of thoracic column from 13 to 12 elements associated with reduction in axial length by one segment [for this occurred at 6 (58)]. Elongation of pubic rami and femoral neck. Posterior relocation of third trochanter and emergence of true hypotrochanteric fossa. Elevation of quadriceps attachments to form “true” linea aspera, signaling fundamental shift in knee extensor/hip extensor proportions conducive to primary propulsion by quadriceps. Probable emergence of tibial dominant knee and transverse tibial plafond (or these occurred at 6). Expansion of fibularis longus attachment to include markedly remodeled medial cuneiform and permanent adduction of great toe, elevation of sustentaculum tali to create mediolateral and longitudinal plantar arches, likely development of “spring ligament,” marked inflation of calcaneal tuber (with secondary introduction of distinct lateral plantar process) for energy absorption at heel strike, gracilization of second metatarsal base, relocation of fibularis longus tendon to more proximo-plantar location (with inferred attendant change in short and long plantar ligaments [see (47)] to support novel transverse arch during toe-off and foot-flat, introduction of “dual phase” metatarsalfulcrumation (addition of transverse axis to oblique axis of fulcrumation). Dorsalization and expansion of capitate head and broadening of trapezoid for greater palmar span, slight reduction in dorsal mobility of Mc5/hamate joint, anterior relocation and near elimination of collateral ligament “grooves” for metacarpophalangeal joint.

8. Hominid clade, Plio-Pleistocene. Elongation of lower limb, global modification of pelvis to expand birth canal (late) including abbreviation of femoral neck and pubic rami. Reduction of modal lumbar column by one (from six to five typically by sacralization of most caudal lumbar). Slight reduction in glenoid angulation of scapula, increased robusticity of thumb, transfer of styloid body from capitate to third metacarpal, palmar rotation of hamulus, loss of growth plate from pisiform, increased robusticity of terminal phalangeal tufts in carpus. Substantial abbreviation of posterior metacarpus, antebrachium, and carpal phalanges. Substantial anteroposterior thickening of navicular and length and eccentricity of calcaneal process of cuboid. Increased robusticity of Mt1. Reduction in frequency of calcification of os peroneum, abbreviation of tarsal phalanges—especially intermediates.

fossils. Contemporary morphogenetics now show that organisms as diverse as sticklebacks and fruit flies can display remarkable parallel evolution merely because they share fundamentally similar genomic toolkits (73, 74). Knuckle-walking in chimpanzees and gorillas appears now to be yet one more example of this phenomenon.

In retrospect, it is impressive that the straightforward cogency of Schultz and the detailed dissections of Straus more accurately predicted the early course of human evolution than the more objective quantitative and technologically enhanced approaches heralded in the last quarter of the 20th century. Recent work in genetics and developmental biology has identified fundamental mechanisms by which morphological structures emerge during evolution. In the study of fossils, such insights have had their primary value as heuristic guides with which to construct and test hypotheses. Understanding the morphogenesis underlying profound shifts in the hominoid bauplan evidenced by *Ar. ramidus* may take years, perhaps even decades, but is likely to further transform our understanding of human natural history.

Ardipithecus has thus illuminated not only our own ancestry, but also that of our closest living relatives. It therefore serves as further confirmation of Darwin's prescience: that we are only one terminal twig in the tree of life, and that our own fossil record will provide revealing and unexpected insights into the evolutionary emergence not only of ourselves, but also of our closest neighbors in its crown.

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4 May 2009; accepted 31 August 2009
10.1126/science.1175833

Quantum Simulators

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Quantum simulators are controllable quantum systems that can be used to simulate other quantum systems. Being able to tackle problems that are intractable on classical computers, quantum simulators would provide a means of exploring new physical phenomena. We present an overview of how quantum simulators may become a reality in the near future as the required technologies are now within reach. Quantum simulators, relying on the coherent control of neutral atoms, ions, photons, or electrons, would allow studying problems in various fields including condensed-matter physics, high-energy physics, cosmology, atomic physics, and quantum chemistry.

More than a quarter of a century after Richard Feynman envisioned a quantum mechanical device for the efficient simulation of quantum systems (1), quantum simulators are now attracting increasing interest in many areas of physics (2). The level of coherent control of quantum systems necessary for the physical realization of quantum simulation is now within reach (3).

The motivation for building a quantum simulator is twofold: It would be very useful for a vast array of problems in physics, chemistry, and biology, and it is feasible with the current technologies. Without the limitations encountered by classical computers when simulating quantum mechanics, quantum simulators would be able to tackle difficult quantum many-body problems. Quantum simulators would not only provide new results that cannot be otherwise predicted or classically simulated, but they would also allow us to test various models. For instance, controllable versions of magnified lattice structures of “solids” (realized with atoms, ions, or electrons) could be used to study difficult problems in condensed-matter physics, such as correlated electrons or quantum magnetism. Moreover, in general, quantum simulations do not require either explicit quantum gates or error correction, and less accuracy is needed. Thus, quantum simulation is typically less demanding than quantum computation. Even with tens of qubits (4–6), one could already perform useful quantum simulations, whereas thousands of qubits would be required for factorizing even modest numbers using of Shor’s algorithm.

In this review, we wish to highlight the progress that has been made so far and pinpoint some future directions as well as discuss the challenges and expectations related to quantum simulators.

Simulating Quantum Systems with Computers

The direct simulation of quantum systems on classical computers is very difficult because of

the huge amount of memory required to store the explicit state of the quantum system. This is due to the fact that quantum states are described by a number of parameters that grows exponentially with the system size. Furthermore, simulating the system evolution requires a number of operations that also increases exponentially with the size of the system. Take, for instance, N spin-1/2 particles; then 2^N numbers must be stored in memory, and a 2^N by 2^N matrix has to be exponentiated to calculate the time evolution of this system. To avoid this “exponential explosion” (1), classic stochastic methods have been developed, but, unfortunately, in many practical situations they fail because of the so-called sign problem (i.e., sampling with nonpositive weight functions). The alternative approach suggested by Feynman is to have “one controllable quantum system simulate another” (1). This idea is appealing because it would solve both the problem of storing the quantum state and simulating its evolution without the exponential explosion or other intrinsic limitations.

Controllable Quantum Systems As Simulators

The generic quantum simulation procedure can be stated as follows: After preparing an initial state, obtain the final quantum state after a certain time evolution, and measure some quantity of interest. This is not easy to achieve because all these steps must be realized with polynomial resources. Consider the initial state preparation and final measurement. In most cases, the preparation of the initial state is difficult. However, for particular cases of interest (i.e., most of the commonly used chemical wave functions or arbitrary pure and mixed many-particle states on a lattice) efficient state preparation protocols exist (7–9). Because quantum state tomography is costly (10), for measurements it would be desirable to directly estimate certain physical quantities like correlation functions or spectra of operators (8, 11). Note that extracting the desired information from the quantum simulator is not always easy. For instance, sometimes the desired information can only be derived indirectly from the measurable quantities of the simulator. Measurement is as crucial as the efficient

simulation of the time evolution of the quantum system.

Analog and Digital Quantum Simulators

How does one quantum system simulate another? One way would be to map the evolution of the system to be simulated onto the controlled evolution of the quantum simulator. Thus, one quantum system would mimic the evolution of another [i.e., a quantum emulator; “...there is to be an exact simulation, that the computer will do exactly the same as nature” (1)]. Such a device will be referred to here as an analog quantum simulator (AQS) (6, 12–14). Another approach would be to use qubits to encode the state of the quantum system, “translate” its unitary evolution in terms of elementary quantum gates, and implement them in a circuit-based quantum computer. This can be regarded as a quantum algorithm for the physical model. We will call this circuit-based simulator a digital quantum simulator (DQS) [see, e.g., (4, 7, 8)].

In analog quantum simulators, the Hamiltonian of the system to be simulated, H_{sys} , is mapped onto the Hamiltonian of the simulator, H_{sim} , which can be controlled to some extent. This can be done when the system and simulator are sufficiently similar, and because of this an AQS would be a dedicated device restricted to simulating a limited class of quantum systems. Moreover, the accuracy of the simulation depends on the degree to which the simulator is able to reproduce the dynamics of the system to be simulated. AQSs are usually emulating an effective many-body theory of the simulated system, so they are limited by the extent to which the theory correctly captures the key physical features of the real system. If the model is incomplete, no matter how good the simulation (i.e., no implementation errors), it will still fail to provide meaningful results about the system being simulated. Two intuitive examples illustrating how analog quantum simulation is achieved are provided in Fig. 1.

In general, the goal of digital quantum simulation is to obtain $|\psi(t)\rangle = e^{-iH_{\text{sys}}t}|\psi(0)\rangle$, the solution of the Schrödinger equation for the time-independent Hamiltonian, H_{sys} , which can be written as a sum of many local interactions. $U = e^{-iH_{\text{sys}}t}$ can be approximated by using several exponentials $e^{-iH_{\text{li}}t}$, where the H_{li} term are the Hamiltonians of the local interactions. In other words, a quantum circuit consisting of one- and two-qubit gates (two-body interactions) for the unitary transformation, U , is constructed. Such a circuit can, in principle, efficiently simulate any finite-dimensional local Hamiltonian. The main advantage of the DQS is this universality. However, the generation of many-body interactions using two-body interactions is by no means an easy problem. Several methods have been developed (15, 16), but this still remains a challenge. The precision (i.e., the desired

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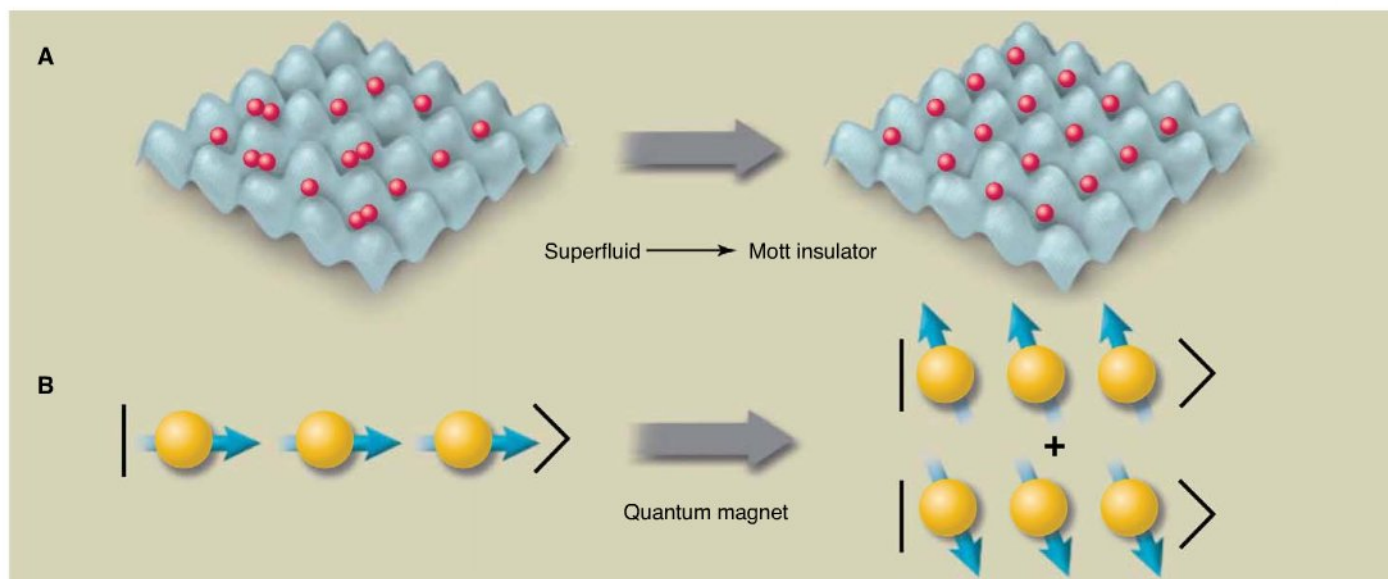


Fig. 1. Examples of analog quantum simulation of quantum phase transitions using ultracold neutral atoms (A) and trapped ions (B). (A) The schematics of the quantum phase transition from a superfluid to a Mott insulator phase realized in (12) by using rubidium atoms trapped in an optical lattice. The ratio between the tunneling energy and the on-site interaction energy was controlled by adjusting the lattice potential depth such that the quantum phase transition could take place. There are alternative

ways of simulating this quantum phase transition with arrays of cavities (21) or arrays of Josephson junctions (27). (B) Magnetic quantum phase transition simulated in (6) using trapped calcium ions. The interactions of individual spins were realized by coupling the internal levels (representing the spin-1/2 states) with a resonant RF field, whereas the spin-spin interactions were simulated by using a state-dependent optical dipole force implemented by a walking wave.

number of bits in the final answer) of DQS can be arbitrarily high; however, this is costly because the required number of quantum gates scales exponentially with the precision of the answer (17).

A DQS is not restricted to recreating the unitary evolution of the system, but it also includes efficient quantum algorithms [e.g., phase estimation for computing eigenvalues (18) or algorithms for computing partition functions (19)]. In some instances, this approach may prove more efficient than directly trying to simulate the unitary time evolution.

Although in the long run the goal would be to build a universal, all-mighty quantum simulator (i.e., a DQS), in terms of practical implementation in the near future, AQS has the advantage. For this reason, most research groups studying quantum simulators are currently investigating AQS, and therefore this trend is reflected in our review.

Applications

Quantum simulators would be able to emulate far larger quantum systems than classical computers. Moreover, being quantum systems themselves, quantum simulators would be able to provide insight on quantum phenomena. Therefore, they are best suited for problems that are intractable on classical computers and those for which more direct experimental studies are very difficult or impossible. Quantum simulators could help tackling difficult problems in condensed-matter physics, most notably quantum phase transitions (Fig. 1), quantum magnetism,

or high-temperature superconductivity. Quantum simulators would also have applications in high-energy physics, the simulation of analog cosmological models, as well as in chemistry. As practical quantum simulators become available, more disciplines might add quantum simulation to their toolbox. Table S1 summarizes some of the proposed applications, as well as the physical systems in which they could be implemented.

Building a Quantum Simulator

Building an AQS requires a controllable quantum mechanical system that can mimic (emulate) the evolution of other quantum systems. However, to reproduce the dynamics of any quantum system, one would need a DQS, which is the yet-to-be-built quantum computer. As experience has shown, such a device is rather difficult to make. However, designing an AQS for a specific problem or a certain class of problems is a much simpler task.

As an example, the study of many-body problems in condensed-matter physics could be achieved with an AQS consisting of an array of qubits together with control fields. A simulator of this kind could be realized with atoms in optical lattices (2, 20), atoms in arrays of cavities (21, 22), arrays of trapped ions (23–25), quantum dots (13, 26), superconducting circuits (27, 28), or electrons trapped on the surface of liquid helium (29, 30) (Fig. 2). The controls of the quantum simulator vary from system to system. They could be laser pulses, radio frequency (RF) pulses, or electric or mag-

netic fields. Next, let us look at some potential quantum simulators.

Atoms and Photons

Neutral atoms in optical lattices. Atoms in optical lattices are very well suited for mimicking condensed-matter physics, as discussed in detail in two recent reviews (2, 20).

Optical lattices can be used for implementing both DQS and AQS. The dimensionality of the lattice can be changed, and various lattice geometries can be obtained by manipulating the optical potential. Moreover, optical lattices are flexible and provide several controllable parameters such as tunneling, on-site interactions, next-neighbor, long-range and multiparticle interactions, external potentials, and Rabi transitions. Spin models can be simulated in a very similar manner as in ion traps. For instance, for optical lattices the interaction between two atoms could be achieved by selectively displacing the optical lattices, whereas in the case of trapped ions the interaction could be realized by pushing the ions with a state-dependent force. In the experiment realizing the quantum phase transition from a superfluid to a Mott insulator (12) (Fig. 1A), the ratio between the tunneling and on-site interaction energies was controlled by adjusting the depth of the optical lattice, but it should also be possible to control the atom-atom interactions via Feshbach resonances (31). So far, addressing individual atoms in optical lattices has been difficult because the separation between neighboring trapping sites is smaller than the best achievable focusing width of the laser beams,

but very recent results show that this technical issue can be solved (32).

Arrays of cavities. Atoms in arrays of cavities could be an alternative to optical lattices (21, 22). In this approach an array of cavities in an arbitrary geometry (Fig. 2), where each cavity interacts with an ensemble of atoms driven by an external laser, is used. The atoms trapped in the cavity together with the photons form polaritons. This system provides a way of simulating the Bose-Hubbard model and quantum phase transitions and allows for the manipulation and measurement of the properties of individual constituent particles. Proposals also suggest measurements and feedback control as tools for realizing quantum simulations (33).

Ions

Trapped ions are another good candidate for implementing a practical quantum simulator. An early experiment investigated nonlinear interferometers (34), and, recently, the transition from paramagnetic to ferromagnetic order has been realized experimentally (6) (Fig. 1B). Trapped ions could be used to study some problems in condensed-matter

physics and to realize even more exotic simulations, such as high-energy physics or cosmology.

Ion trap quantum simulators are quite flexible and allow the implementation of both DQS and AQS. Moreover, one can exploit both the internal energy levels and the vibrational modes of the trapped ions. Coherent control can be realized with high fidelity (3), so the scalability to many ions is the major challenge for ion trap quantum simulators. Several solutions may be available: using long strings of ions, planar Coulomb crystals (23), or arrays of microtraps (24) or it could also be possible to trap ions in optical lattices as suggested in (25). The two-qubit interactions are usually realized with optical forces, but a method for laserless simulation (avoiding the scattering problem) with ions in arrays of microtraps has been proposed (24). A substantial advantage of trapped ions is the ease of measuring and manipulating individual ions. Such a feature is not available in typical condensed-matter systems.

Electrons

Quantum dots. Arrays of semiconducting quantum dots can be realized in two-dimensional

electron gas with superposed two-dimensional mesh gates (13, 26). The material of choice is usually GaAs. By adjusting the mesh gate design and voltage, various lattice geometries can be realized. Other types of quantum dots can be introduced in the semiconductor during growth (i.e., small islands of InGaAs within a GaAs matrix). In these quantum dots, one can make use of optical transitions. In quantum dot arrays, the Fermi-Hubbard model (26) or the CuO plane in high-temperature superconductors (13) could be simulated. Using quantum dots may provide an advantage over atoms in optical lattices because of the very low temperatures relative to the Fermi temperature that can be reached and the natural long-range Coulomb interaction (26). An interesting feature of quantum dots is that they behave like “artificial atoms,” and coupled quantum dots can be seen as “artificial molecules” so they can be used for the analog simulation of chemical reactions (14).

Superconducting circuits. Superconducting circuits can also behave like “artificial atoms,” so they can be used to test quantum mechanics at macroscopic scales and conduct atomic phys-

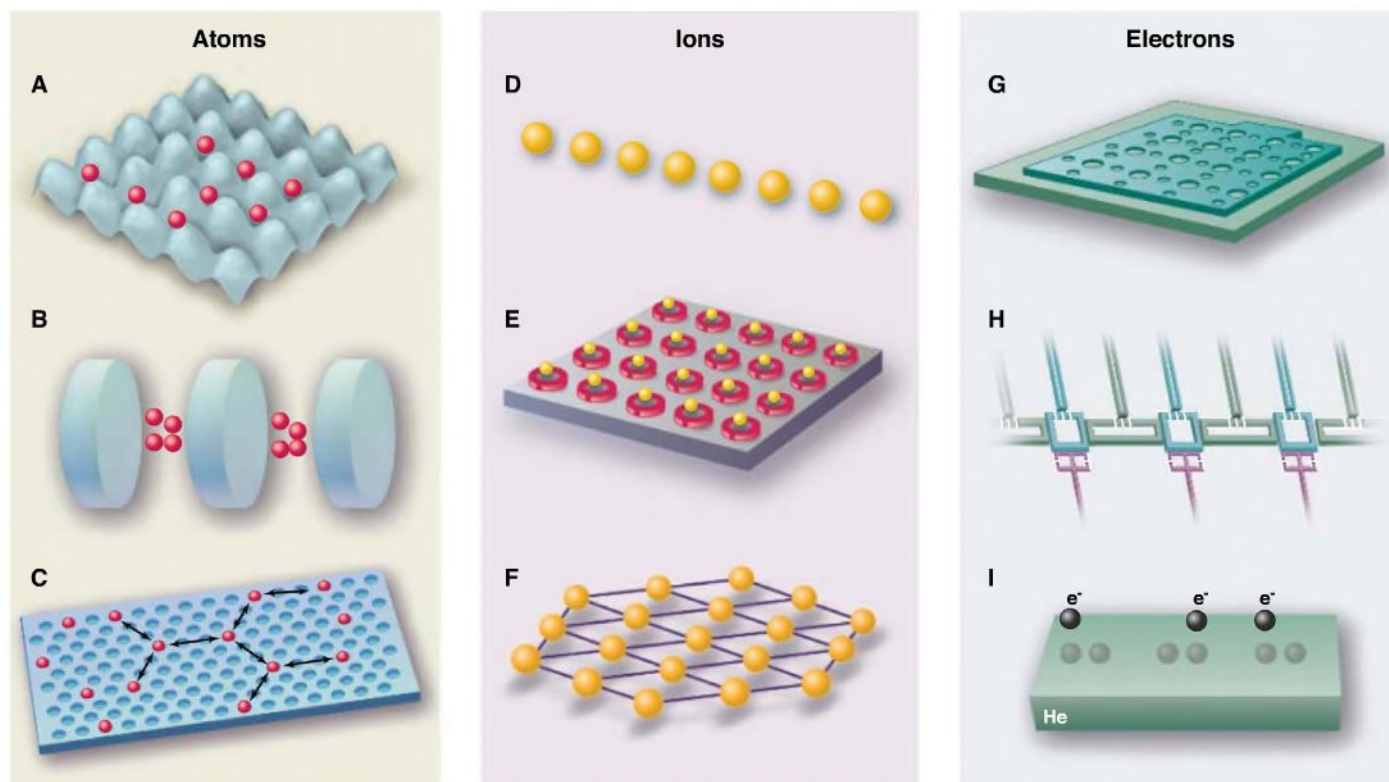


Fig. 2. One-dimensional or 2D arrays of qubits plus controls could be used to simulate various models in condensed-matter physics. Examples of physical systems that could implement such analog quantum simulations include the following: atoms in optical lattices (20) (A) or in 1D (B) or 2D (C) arrays of cavities (21, 22); ions in linear ion chains (D), 2D arrays of planar traps (24) (E), or 2D Coulomb crystals (23) (F); or electrons in quantum dot arrays created by a 2D mesh (13, 26) (G), or arrays of superconducting circuits (28) (H), or trapped on the surface of liquid helium (30) (I). The average distance between the atoms is, in the case of optical lattices, less than 1 μm ; in 2D arrays of cavities, it would scale as the ratio between the wavelength and the refractive index. As for the interior distances in ion trap arrays, they should be

about 10 to 50 μm and about the same for 2D Coulomb crystals. In arrays of quantum dots, the spacing between dots is about 0.1 μm . In superconducting circuits, the distance between junctions can be less than a micrometer. In the case of electrons on helium the distance between neighboring sites would be about 1 μm . These interqubit distances (from 0.1 to 10 μm) should be compared with the far smaller average interatomic distances in solids, which are ≤ 1 nm. The systems shown above realize a 1- or 2D array of qubits, which can be manipulated in different manners. The larger distances between qubits make quantum simulators more controllable and easier to measure. Therefore, they can be thought of as toy models of the magnified lattice structure of a “solid,” with a magnification factor of three orders of magnitude.

ics experiments on a silicon chip (3, 35). There is a deep analogy between natural atoms and the artificial atoms composed of electrons confined in small superconducting islands. Whereas natural atoms are driven by using visible or microwave photons, the artificial atoms in the circuits are driven by currents, voltages, and microwave photons. The effects of electric and magnetic fields on the circuits are the analogs of the Stark and Zeeman effects in atoms. Superconducting circuits as artificial atoms can be used to simulate atomic physics and quantum optics (35). Furthermore, early experiments have observed a one-dimensional (1D) Mott insulator formed by quantum vortices in arrays of Josephson junctions (27). Other applications are summarized in table S1.

A limitation of microfabricated solid state qubits is the difficulty of producing them with high uniformity. This can yet turn into an advantage when modeling condensed-matter systems, where defects and disorder are often crucially important. Furthermore, the uniformity of solid state qubits has been increasing in time and should be less of a problem in the future.

We also mention that electrons trapped on the surface of liquid helium could be used for simulating spin models (29, 30).

Others

The quantum simulators discussed so far are 1D or 2D arrays of qubits plus controls as illustrated in Fig. 2. Nuclear spins in organic molecules manipulated with nuclear magnetic resonance (NMR) techniques have been used for quantum simulation (especially DQS). In one of the first quantum simulation experiments, the dynamics of truncated quantum harmonic and anharmonic oscillators have been simulated (36). More recently, the pairing Hamiltonian has been simulated by using NMR (17). Solid-state NMR has been proposed for simulating the phase transition from a paramagnetic to antiferromagnetic phase (37). Although liquid-state NMR is not scalable, experiments on spin diffusion in solid state systems can be thought as large-scale AQS, and therefore, NMR may be used for quantum simulation.

Linear optics has been used to implement some quantum simulations (table S1).

Decoherence and Limitations

Although quantum simulators are affected by the interactions with the environment in the same way as quantum computers, decoherence is not such a big problem because in quantum simulations only limited precision is required. Moreover, it was suggested that the decoherence of the simulator might be useful (4) because it could serve as a rough way of modeling the decoherence of the simulated system. In (38), it was demonstrated through calculations and an NMR experiment that it is indeed possible to exploit the natural decoherence of the simulator, and with an appropriate choice of the mapping

between the system and simulator, one may take advantage of the natural symmetries in order to modify the effective decoherence of the simulator. However, there are certain limitations. The simulated system does not necessarily decohere in a similar way as the simulator (39), and, therefore, one should be cautious when trying to include decoherence in the simulation. To what extent one can make use of decoherence in the simulations depends on the type of decoherence, the simulated system, and the specific way the system is mapped onto the simulator. Note that the simulation of quantum open systems does not necessarily require the inclusion of the decoherence of the simulator (40).

The errors in quantum simulation are usually taken too lightly. Indeed, the required level of precision and control is much lower than for quantum computation; however, errors still need to be minimized. Recently, the effect of noise in two-body interactions and local control operations used for the simulation of many-body interaction Hamiltonians has been investigated in detail (16). However, this problem clearly needs more attention.

Challenges and Prospects

Recent theoretical and experimental results on quantum simulation are quite encouraging, and it seems that in the near future practical quantum simulators might be built. However, there still are some problems to be overcome. From the experimental point of view, improved controllability and scalability should be realized. Besides optical lattices, other systems cannot yet handle large arrays of qubits. However, even with a relatively small quantum simulator, various interesting physical regimes could be explored.

Further theoretical studies of decoherence and control would be useful. For each physical system, the minimum requirements for realizing useful quantum simulations and the potential limitations have to be investigated further. Moreover, new applications of quantum simulation should be explored.

Finally, two interesting directions closely related to quantum simulation should be mentioned: One is the study of entanglement in many-body systems and its relation with quantum phase transitions; the other is the development of classical numerical algorithms, inspired by the methods in quantum information and computation, for the simulation of quantum many-body systems.

Conclusions

Considerable progress toward building a quantum simulator has been achieved in the past decade, and in the near future we might witness the first practical applications. Surely, Feynman (1) would be very pleased by these results and promising perspectives, especially the first experimental demonstration of benchmark quantum simulations with tens of qubits. Quantum simulators would have tremendous impact in

many fields, providing a means of exploring new physical phenomena.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/108/DC1

Table S1

References

10.1126/science.1177838

Global Surface Wave Tomography Using Seismic Hum

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Earth's background free oscillations, or seismic "hum" (1–3), are excited continuously and persistently by the ocean and atmosphere. Cross-correlation (CC) analysis of hum signals shows a clear global propagation of back-

distance should indicate clear Rayleigh wave propagation (5), and the CC function should exhibit Green's-function-like signals at a station when a point source of excitation exists at the other station (6). This is the case in our data (Fig. 1A). The sym-

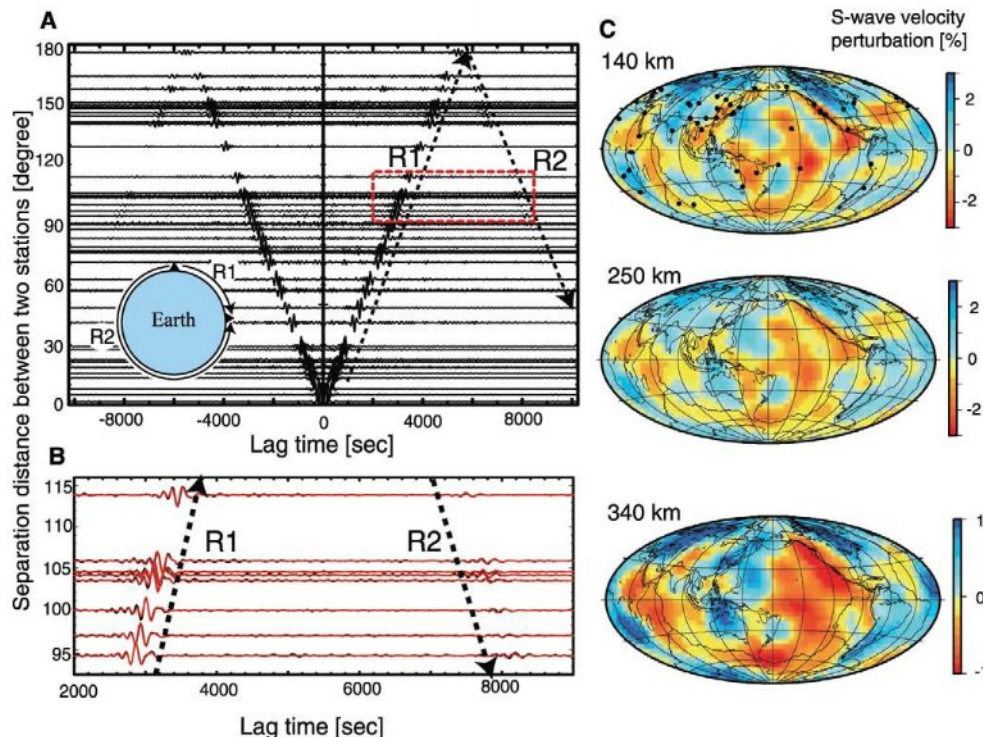


Fig. 1. (A) Observed CC functions plotted against separation distance between a pair of stations. They show R1 (Rayleigh wave traveling along the minor great circle arc) arrivals and R2 (traveling along the major great circle arc) arrivals. Positive lag time is defined as time advance of the second seismogram. (Inset) Schematic figure of R1 and R2. (B) Comparison of observed (black) and synthetic (red) CC functions shown for an enlarged view of the red-dotted box in (A). (C) S-wave velocity structure at depths from 140 to 340 km. We also show station location used in this study by circles.

ground Rayleigh waves, which opens the possibility for global surface wave tomography without referring to earthquakes.

One technique known as ambient noise tomography (4) cross-correlates random surface waves (Love and Rayleigh waves) with periods of around 10 s excited by ocean swells. Here, we show that the randomness of the sources of both the hum and ambient noise allows this technique to be applied for hum at much longer periods, 100 to 400 s, to explore the mantle to depths of 500 km.

We analyzed continuous records for 1986 to 2003 observed at 54 stations of the International Federation of Digital Seismographic Networks (FDSN). The record section of the CC functions between two stations as a function of their separation

metry of the functions for positive and negative time lags is an indication of the randomness of the hum sources.

Following (5), we calculated synthetic CC functions for a global one-dimensional (1D) model [the Preliminary Reference Earth Model (PREM) (7)] (Fig. 1B), and these generally agree with the observations except for a small discrepancy, which we attribute to lateral heterogeneity of Earth not to source heterogeneity (8).

We measured the phase differences between the observed and synthetic CC functions of 906 R1 and 777 R2 Rayleigh waves at six central periods: 376, 323, 275, 233, 172, and 121 s. We isolated the R1 and R2 wave packets along their group velocity curves by using 2000-s time windows with the

central time predicted from the group velocity. These data were inverted to obtain isotropic phase-velocity maps of the Rayleigh waves with 5° by 5° grid points at each central period (8, 9).

The phase-velocity maps were inverted to obtain a 3D S-wave velocity model (Fig. 1C) that includes a correction for the crustal contribution (8). The model at 140 km shows the typical low-velocity anomalies beneath plate boundaries and high-velocity anomalies beneath old continental cratons in Asia, North and South America, and Australia. Our model compares well with other global tomographic models created with use of earthquakes (8). A good agreement is obtained at

all depths of the upper mantle, including the transition zone, which demonstrates that robust deep 3D structure of Earth can be recovered from seismic hum.

Our tomographic approach could conceivably be used in planetary exploration for investigating the deep internal structures of Mars or other bodies. Martian atmospheric disturbances might excite background long-period Rayleigh waves (2, 10, 11), which might then be used to retrieve Green's-function-like signals between stations of a small Martian seismic network (8).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/112/DC1

Materials and Methods

Figs. S1 to S4

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15 May 2009; accepted 24 August 2009

10.1126/science.1176389

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Fluxonium: Single Cooper-Pair Circuit Free of Charge Offsets

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The promise of single Cooper-pair quantum circuits based on tunnel junctions for metrology and quantum information applications is severely limited by the influence of offset charges: random, slowly drifting microscopic charges inherent in many solid-state systems. By shunting a small junction with the Josephson kinetic inductance of a series array of large-capacitance tunnel junctions, thereby ensuring that all superconducting islands are connected to the circuit by at least one large junction, we have realized a new superconducting artificial atom that is totally insensitive to offset charges. Yet its energy levels manifest the anharmonic structure associated with single Cooper-pair effects, a useful component for solid-state quantum computation.

Electric charge can be manipulated at the level of a single charge quantum (I) in two types of superconducting circuits with different topologies. The minimal example of the first type of circuit is the Cooper-pair box, which consists of an isolated superconducting electrode (an “island”) connected to a superconducting reservoir on one side by a small tunnel junction, and on the other side by a gate capacitance in series with a voltage source. The dynamics of the island is described by two variables: the integer number of Cooper pairs occupying the island and its conjugate, the 2π -cyclic superconducting phase difference between the island and the reservoir. The junction area must be sufficiently small that the electrostatic energy of the island due to an extra Cooper pair is larger than the Josephson energy of its coupling to the reservoir, thus confining fluctuations of the number of Cooper pairs below unity. Stated in electrical engineering language, one needs $Z_J > R_Q$, where the junction reactive impedance $Z_J = (L_J/C_J)^{1/2}$ is defined by the Josephson characteristic inductance L_J and capacitance C_J (2), and where the superconducting impedance quantum is given by $R_Q = \hbar/(2e)^2 \approx 1 \text{ k}\Omega$, denoting Planck's constant $\hbar$ and the charge quantum e . The second type of circuit is based on a superconducting loop connecting the two electrodes of a small junction with an inductance that exceeds L_J . The circuit conjugate variables are now the magnetic flux generated by the persistent current in the loop and the displacement charge on the plates of the small junction capacitance. When $Z_J > R_Q$, the large loop inductance is submitted to quantum fluctuations of flux larger than the flux quantum $\Phi_0 = 2\pi\hbar/2e$; and therefore, according to the Heisenberg principle, the junction charge fluctuations are reduced below the value $2e$.

In practice, the realization of both circuit types faces fundamental difficulties. Islands are ex-

posed to random electric fields due to fluctuating charged impurities, which are ubiquitous in most solid-state environments and whose compounded effect is described by a noisy offset charge. Although the fully developed charging effects were demonstrated for the Cooper-pair box (3, 4), it soon became clear that the low-frequency offset charge noise was a major source of decoherence for charge qubits derived from this device (4–7). This state of affairs has prompted the development of alternative superconducting qubits based on large junctions with $Z_J \ll R_Q$, avoiding the single Cooper-pair regime and the related charge offset problem (8–10). On the other hand, implementing the island-free circuit, which is immune to charge offset noise, is another hard problem. This is because any finite-length wire with inductance L always comes with self-capacitance C , which reduces the total charging energy of the circuit and therefore steers it away from the charging regime, unless $(L/C)^{1/2} \gg R_Q$. In fact, a purely electromagnetic inductance is incompatible with the single Cooper-pair effects, because $(L/C)^{1/2}$ is then bounded by the vacuum impedance $(\mu_0/\epsilon_0)^{1/2} \approx 377 \text{ }\Omega < R_Q$, μ_0 and ϵ_0 being vacuum permeability and permittivity (11, 12).

In this paper, we present experimental results from a novel single Cooper-pair circuit based on a superconducting loop, which solves both the inductance and the offset charge noise problems. The small junction of our circuit is shunted by a series array of carefully chosen larger-area tunnel junctions (Fig. 1, A to C). Here, all islands are connected to the rest of the circuit by at least one large junction, so that quasistatic offset charges on all islands are screened. The large capacitances of the array junctions prevent phase slips within the array, and at excitations whose frequencies are below the junction plasma frequency, the array effectively behaves as an inductive wire. By choosing a sufficiently large number of array junctions, it is possible to create an inductance exceeding that of the small junction. At low energies, the loop is effectively described by the loop flux $\hat{\Phi}$ and the small junction charge $\hat{Q}$, satisfying $[\hat{\Phi}, \hat{Q}] = i\hbar$.

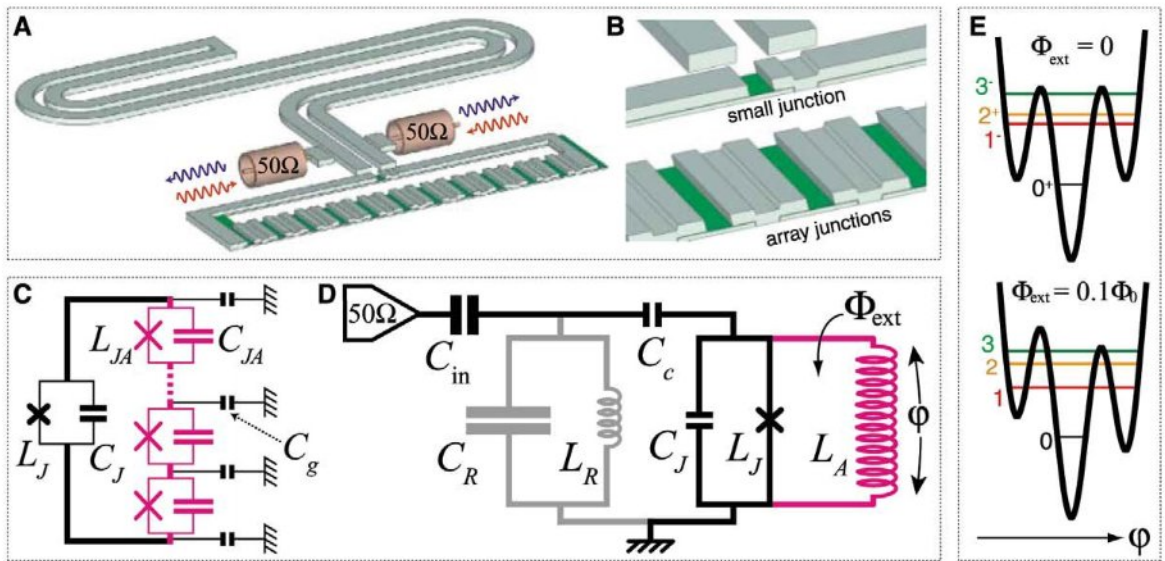
To form a charge offset-free, inductively shunted junction, four conditions involving the effective inductance L_{JA} and capacitance C_{JA} of the N array junctions are required: (i) $NL_{JA} \gg L_J$, (ii) $e^{-8R_Q/Z_{JA}} < \epsilon \ll 1$, (iii) $Ne^{-8R_Q/Z_{JA}} \ll e^{-8R_Q/Z_J}$, and (iv) $N < (C_{JA}/C_g)^{1/2}$. In the relation (i), we simply estimate the total array inductance to be NL_{JA} and require that it exceed the small junction inductance, allowing it to support the large flux fluctuations of the loop. The relation (ii), where $Z_{JA} = (L_{JA}/C_{JA})^{1/2}$ is the array junction reactive impedance, dictates the minimum size of the array junctions necessary to reduce (13) the uncontrolled offset charge on the islands of the circuit below the desired value on the order of $2e \times \epsilon$. The relation (iii) ensures that the inductive role of the array is not jeopardized by quantum phase slips (14). Specifically, the probability amplitude of a phase slip event within the array (l.h.s.) must be negligible compared to that in the small junction (r.h.s.). According to relation (iii), a fluxon tunnels in and out of the loop predominantly via the small junction, thus effectively erasing the discrete character of the array. Lastly, relation (iv) states that the inductance of the array is not shunted by the parasitic capacitances C_g of array islands to ground. It is obtained by estimating the array parasitic resonance frequency to be $(L_{JA}N \times C_g N)^{-1/2}$, and requiring that it be larger than the junction plasma frequency $(L_J C_{JA})^{-1/2}$. It is relation (iv) which, with present junction technology, most severely limits the maximum number of junctions in the array and thus its maximum inductance.

We have implemented the above array proposal and constructed a new superconducting artificial atom which we have nicknamed fluxonium. It contains $N = 43$ Al/Al oxide/Al Josephson junctions (15), so that $Z_{JA} \approx 0.5 R_Q$ and a small junction with $Z_J \approx 1.5 R_Q$ (16). The above four conditions being realized, the fluxonium can be modeled (Fig. 1D) as a small junction shunted by an inductance L_A (17). The three characteristic energies of this model, namely $E_L = (\Phi_0/2\pi)^2/L_A$, $E_J = (\Phi_0/2\pi)^2/L_J$, and $E_C = e^2/(2C_J)$, have values corresponding to 0.52, 9.0, and 2.5 GHz, respectively. The additional $L_R C_R$ resonator, capacitively connected to the small junction (Fig. 1D), reads out the atom in a manner analogous to the dispersive measurement of circuit quantum electrodynamics (cQED) qubits (18). It is implemented by a quarter-wave superconducting coupled microstrip resonator (Fig. 1A) with quality factor of 400, due to capacitive coupling to the two 50- Ω measurement ports. The resonator frequency $\omega_R = (L_R C_R)^{-1/2} \approx 2\pi \times 8.17 \text{ GHz}$ is pulled by the reactance of the fluxonium circuit and is monitored by a standard ultra-low-noise microwave reflection technique. The fluxonium reactance depends on its quantum state, an effect leading to a purely dispersive state measurement (15). An externally imposed, static magnetic flux Φ_{ext} threading the loop Φ_0 periodically modulates the spacings of energy levels of our artificial atom.

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Fig. 1. (A) Sketch of a small Josephson junction shunted by an array of larger-area junctions. The two superconducting leads of the small junction are coupled capacitively to a quarter-wave microwave resonator, a parallel wire transmission line shorted on the opposite end. The resonator itself is probed capacitively and symmetrically via two 50- Ω microwave ports, resulting in a quality factor of 400. The whole device is made with single-step standard Al/Al oxide/Al double-angle evaporation through an e-beam lithography mask on a high-resistivity Si substrate. (B) Close-up view of the small junction region, showing top and bottom junction electrodes (gray) and their thin oxide layer (green). Array junctions are about one order of magnitude larger in area and are spaced as tightly as e-beam lithography resolution allows, minimizing microwave parasitics. (C) Electrical circuit representation of the loop formed by the small junction (black), with Josephson inductance L_J and capacitance C_J , shunted by the array of larger junctions (purple), with the corresponding inductance L_{JA} and capacitance C_{JA} . Islands formed between the array junctions have small capacitance to ground C_g . (D) Simplified circuit model of the fluxonium, consisting of three sections: (i) the circuit equivalent of a



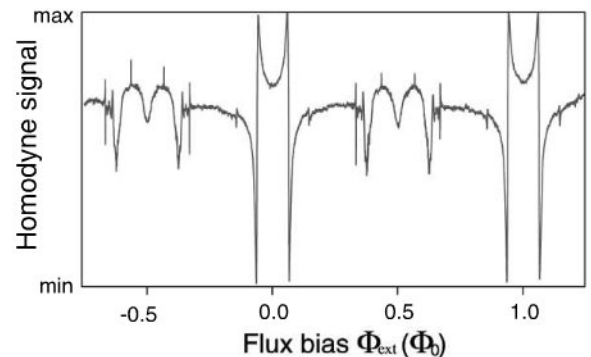
Cooper-pair box, where the small junction with capacitance C_J and nonlinear Josephson inductance L_J is capacitively (with capacitance C_c) coupled to the probe (solid black), so that $L_J/C_J^{1/2} > \hbar/(2e)^2$; (ii) giant inductance $L_A \gg L_J$ provided by the junction array (purple); (iii) a parallel combination of C_R and L_R so that $(L_R/C_R)^{1/2} \approx 50 \, \Omega \ll \hbar/(2e)^2$, which is the circuit model for the distributed transmission line resonator (gray). (E) Potential seen by the reduced flux ϕ and energy spectrum of the circuit (D) for two values of external flux Φ_{ext} . At $\Phi_{\text{ext}} = 0$, energy levels possess well-defined parity as indicated with plus and minus signs next to the level numbers. In contrast with the RF-SQUID or flux qubit, there is on average only one level per local minimum.

Introducing the operators $\hat{N} = \hat{Q}/2e$ and $\hat{\phi} = 2e\Phi/\hbar$, describing the reduced charge on the junction capacitance and its conjugate reduced-flux operator (19), the Hamiltonian of the fluxonium coupled to its readout resonator can be written as

$$4E_C \hat{N}^2 + \frac{1}{2} E_L \hat{\phi}^2 - E_J \cos(\hat{\phi} - 2\pi\Phi_{\text{ext}}/\Phi_0) + g\hat{N}(\hat{a} + \hat{a}^\dagger) + \hbar\omega_R \hat{a}^\dagger \hat{a} \quad (1)$$

Here $\hat{a}$ is the photon annihilation operator for the resonator and g is the atom-resonator coupling constant. The second term and the range of definition of $\hat{\phi}$ and $\hat{N}$, whose eigenvalues are here both on the entire real axis, distinguishes the form of Hamiltonian Eq. 1 from that of the Cooper-pair box in cQED experiments (18). There are three important points to note concerning this Hamiltonian (20): (i) It is invariant under the transformation $\hat{N} \rightarrow \hat{N} + N_{\text{offset}}$ (N_{offset} stands for offset charge value), hence the charge-free character of our device; (ii) it differs from that of the transmon (13), because offset charge influence is screened for all states, not just for the low-lying states; (iii) its second term, despite the fact that E_L is the smallest of the fluxonium energies, has a nonperturbative influence on the full energy spectrum of this artificial atom, which presents strongly anharmonic transitions (21) (Fig. 1E). Our experiment probes these transitions by mi-

Fig. 2. Modulation of the reflected 8.18-GHz microwave signal with externally applied flux Φ_{ext} . The signal is clearly flux-periodic, indicating that the junction ring is closed and superconducting. The values of Φ_{ext} at which the signal undergoes full swings correspond to the anticrossings of the 0–1 transition frequency of the device with the resonator bare frequency, later inferred to be 8.1755 GHz. The measurement tone populates the resonator with less than 0.01 photon on average.



crowave spectroscopy, from which we infer the size of charge fluctuations.

To characterize the fluxonium, we first measured the ground-state resonator pull as a function of Φ_{ext} . The results (Fig. 2) show the expected Φ_0 periodicity as well as the avoided crossings of the resonator frequency and the ground-to-excited-state transitions. This confirms that the entire 44-junction loop is superconducting and that the resonator-atom system is in the strong coupling regime of cavity QED (22).

Next, we performed a two-tone spectroscopy measurement (23) at a fixed flux $\Phi_{\text{ext}} = 0.05 \, \Phi_0$, during which, in addition to the fixed frequency readout tone, we probed the transition frequencies of the atom through a second, variable-frequency spectroscopy tone. The resulting peaks (Fig. 3) correspond to the later-determined 0–1, 0–2, and 0–3 transitions from

the atom ground state. The peaks are well-fitted by Lorentzians, and their power-dependent widths and heights are well explained by the Bloch equations of precessing spin 1/2 (24) (Fig. 3, insets). Extrapolating fitted line widths to zero spectroscopy power, we obtained lower bound estimates of their decoherence time at 350, 250, and 80 ns, respectively.

Our main result is the spectroscopic data collected as a function of both spectroscopy frequency and flux (Fig. 4A). Φ_{ext} variations span 20% of Φ_0 around $\Phi_{\text{ext}} = 0$ instead of the usual 1% or less around $\Phi_0/2$ in flux qubit experiments (9). In Fig. 4B, we compare the measured peak center frequencies with the prediction for the 0–1, 0–2, 0–3, and the two-photon 0–4 transitions obtained from numerical diagonalization of the Hamiltonian (Eq. 1). We are in effect fitting more than three flux-dependent

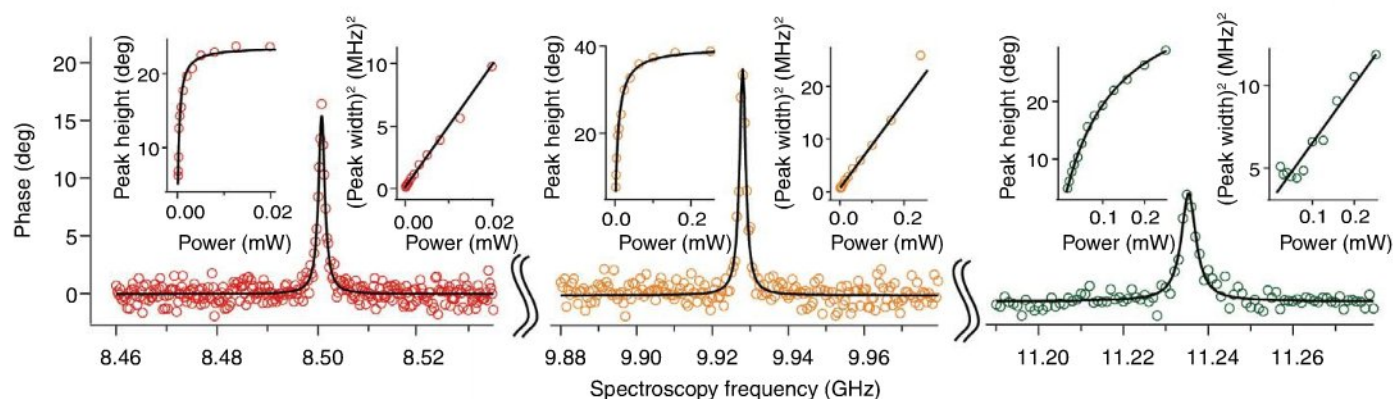
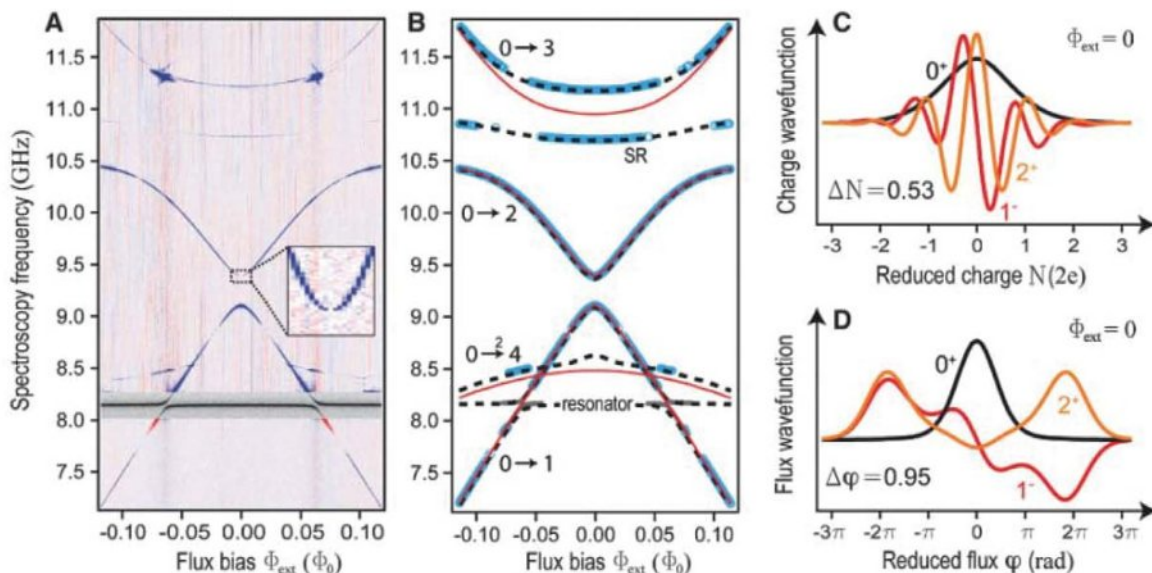


Fig. 3. Phase (colored circles) of reflected readout tone as a function of spectroscopy tone frequency taken at $\Phi_{\text{ext}} = 0.05 \Phi_0$. Data for the first three resonances (further identified as transitions from the ground state to states 1, 2, and 3) are shown from left to right in red, orange, and green, respectively. Resonances are well fitted by Lorentzians (solid black lines) for

a broad range of spectroscopy powers. Insets on the two sides of each resonance show the dependence of the resonant peak height (left) and width squared (right) on the spectroscopy tone power. Data in all insets follow the predictions (solid black lines) of Bloch equations describing relaxation dynamics for a spin 1/2 and indicate that all transitions involve one photon.

Fig. 4. (A) Phase of reflected readout tone as a function of the spectroscopy tone frequency and external flux. The color scale encodes the value of the phase, with zero corresponding to the mauve background, blue to positive values (peaks), and red to negative values (dips). The gray region shows the reflected phase of a single tone, swept close to the resonator bare frequency exhibiting a 50-MHz vacuum Rabi splitting of the resonator with the fluxonium transition 0–1. The inset in (A) zooms in on the central region of the 0–2 transition and confirms that it is indeed symmetry-forbidden at $\Phi_{\text{ext}} = 0$. (B) Measured peak frequencies (blue circles) fitted by the numerically computed spectrum of the Hamiltonian (Eq. 1) (solid red lines) and its modification (see supporting online text) to explain the additional transition labeled SR (dashed black lines). (C) Amplitude of fluxonium wave functions for levels 0 (black), 1 (red), and 2 (orange), computed in charge representation at zero flux bias, using circuit parameters extracted from



the fits. (D) Same as in (C) but in flux representation. The flux representation wave functions demonstrate that the reduced flux is delocalized as compared to the size of the Josephson well, whereas charge wave functions confirm that the localization of charge on the junction is less than a single Cooper-pair charge. In this circuit, the junction charge is a continuous variable, in contrast to the Cooper-pair box, and flux swings of more than 2π are allowed.

functions (the flux-dependent transition frequencies) with only three a priori unknown energies E_C , E_L , and E_J , so the problem is severely overconstrained. The fit of the line (Fig. 4B) labeled SR (for array self-resonance) requires a minor extension of the model, taking into account parasitic capacitances across the array (15). Apart from introducing another resonator mode coupled to the atom, this extension by no means invalidates the inductive character of the array, at least as far as the 0–1 and 0–2 transition of the fluxonium are concerned. Even the perturbation of the 0–3 and 0–4 transition frequencies by this extra mode is less than 2%.

Based on the excellent agreement between theory and experiment, we inferred the wave

functions of the first three energy levels and plotted their amplitudes both in charge (Fig. 4C) and flux (Fig. 4D) representations for $\Phi_{\text{ext}} = 0$. In the ground state, we find that the ratio of charge to flux fluctuations is $\Delta N / \Delta \varphi = 0.56$, about five times smaller than the fine structure constant allows for a conventional resonator. This confirms that the charge in our circuit is indeed localized at the single Cooper-pair level ($\Delta N = 0.53$, $\Delta \varphi = 0.95$). The wave functions in flux representation (Fig. 4D) can be interpreted as simple superpositions of states in which the reduced flux φ is localized in the wells of the Josephson cosine potential (fluxon states, hence the name fluxonium). The parity of fluxonium states, which forbids the 0–2 transition at zero

external flux, manifests itself explicitly by a remarkable "hole" in the corresponding spectroscopic line (Fig. 4A, inset). The allowed transition between the second and third levels is particularly spectacular because it corresponds to motion of the total flux in the fluxonium loop by two whole flux quanta. This is to be contrasted with the 10% of flux quantum or less flux motion involved in transitions of the flux and phase qubits (8, 9). Nevertheless, despite the large flux fluctuations of the system and the corresponding charge pinning, the circuit has complete immunity to offset charge variations: The data of Fig. 4A were taken piecemeal in 72 hours, and no jumps or drifts were observed during this period.

We have thus demonstrated that an array of Josephson junctions with appropriately chosen parameters can perform two functions simultaneously: short-circuit the offset charge variations of a small junction and protect the strong non-linearity of its Josephson inductance from quantum fluctuations. The data show that the array possesses a microwave inductance 10^4 times larger than the geometric inductance of a wire of the same length (20 μm). The reactance of such an inductor is about $20 R_Q \approx 20 \text{ k}\Omega$ at 10 GHz, whereas its resistance is less than 1Ω . The spectrum of the fluxonium qubit suggests that it is as anharmonic as the flux qubit but as insensitive to flux variations as the transmon qubit. Possible applications of this single Cooper-pair charging effect immune to charge noise include the observation of fully developed macroscopic quantum-coherent oscillations between fluxon states (25), the search for Λ or V transition configurations for the shelving of quantum information (26) in superconducting artificial atoms, topological protection of superconducting qubits (27), and, finally, the long-sought quantum metrology of electrical current via Bloch oscillations (28, 29).

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16. Because of coupling of the fluxonium circuit to the readout resonator (Fig. 1D), C_J is renormalized to the value $C_J + C_c$, with corrections on the order of $C_c/C_J \approx 1\%$.
17. Here we acknowledge experiments (30, 31) in which a small Josephson junction was dc-biased in series with a micrometer-scale disordered film resistance exceeding R_K (30) or with an array of small-junction superconducting quantum interference devices (SQUIDs) (31) with zero-bias resistance tuned by magnetic field to exceed R_K . Both experiments aimed at protecting the small junction from the shunting effect of a low-impedance environment, using highly dissipative biasing elements. Although the results of dc current-voltage measurements were consistent with single Cooper-pair effects, they were distorted by Joule heating and other out-of-equilibrium effects in these biasing elements. We avoid the problem of dissipation with an array employing the pure Josephson kinetic inductance.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/113/DC1

Materials and Methods

SOM Text

Fig. S1

Table S1

References

29 April 2009; accepted 28 July 2009

10.1126/science.1175552

Preferential Growth of Single-Walled Carbon Nanotubes with Metallic Conductivity

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Single-walled carbon nanotubes can be classified as either metallic or semiconducting, depending on their conductivity, which is determined by their chirality. Existing synthesis methods cannot controllably grow nanotubes with a specific type of conductivity. By varying the noble gas ambient during thermal annealing of the catalyst, and in combination with oxidative and reductive species, we altered the fraction of tubes with metallic conductivity from one-third of the population to a maximum of 91%. In situ transmission electron microscopy studies reveal that this variation leads to differences in both morphology and coarsening behavior of the nanoparticles that we used to nucleate nanotubes. These catalyst rearrangements demonstrate that there are correlations between catalyst morphology and resulting nanotube electronic structure and indicate that chiral-selective growth may be possible.

Carbon nanotubes have yet to see ubiquitous application in electronic devices, despite their electronic properties (1). This is largely because the electronic properties are related to nanotube bonding configuration (known as its chirality). Though some methods exist to

bias the population of one type of nanotube during synthesis, there is only a limited understanding of exactly what determines chirality during synthesis.

There have been important achievements in separating single-walled carbon nanotubes (SWNTs) according to their conductivity (2–5)

and in enriching the distribution of nanotubes with a specific conductivity (6, 7). Meanwhile, there have been a few reports regarding direct control over nanotube structure during growth (8–10). The fact that SWNTs with narrow chiral distributions have been successfully grown (8) indicates that there may be a specific mechanism that controls chirality. The concept of amplifying existing SWNT distributions by seeding growth from another nanotube with well-defined chirality has been proposed (9); however, evidence for the maintenance of chirality has not yet been reported (10). The preferential growth of nearly 90 (11) to 96% (12) of semiconducting SWNTs by plasma-enhanced chemical vapor deposition has been reported, but the mechanism that leads to this selectivity remains unclear.

In this work, we grew SWNTs from Fe nanocatalysts deposited onto a SiO₂/Si support

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and in situ annealed in a He or Ar ambient that contains various ratios of H_2 and H_2O . We used methane as the carbon source at $860^\circ C$ (13). Scanning electron microscopy studies show that increasing concentration of reductive species during catalyst conditioning from Ar: H_2 (9:1) to Ar: H_2 (8:2) at 840 torr in the presence of ~ 3.5 mtorr of H_2O results in a relatively higher density of SWNTs on the substrate (fig. S1, A and B). In contrast, in the presence of a He-

supported ambient with ~ 3.5 mtorr of H_2O , the density of grown SWNTs is high and does not show strong dependence on H_2 concentration (fig. S1, C and D). Raman spectroscopy analyses of these samples reveal that the ratio of metallic tubes to semiconducting tubes is sensitive to catalyst conditioning history.

The strong variations of nanotube density in different environments led us to perform systematic studies of SWNTs grown on catalysts

that were in situ annealed under different ambients. For a reasonable estimation of the ratio of metallic tubes to semiconducting tubes, we used the integral intensities of the Raman radial breathing modes (RBMs), which we define as $R = I_{met}/I_{sem}$ (here, I_{met} is the intensity of the metallic tubes, and I_{sem} is the intensity of the semiconducting tubes) (2). We used three excitation wavelengths ($\lambda = 785$, 632.8, and 532 nm) in our experiment. Of these, $\lambda = 632.8$

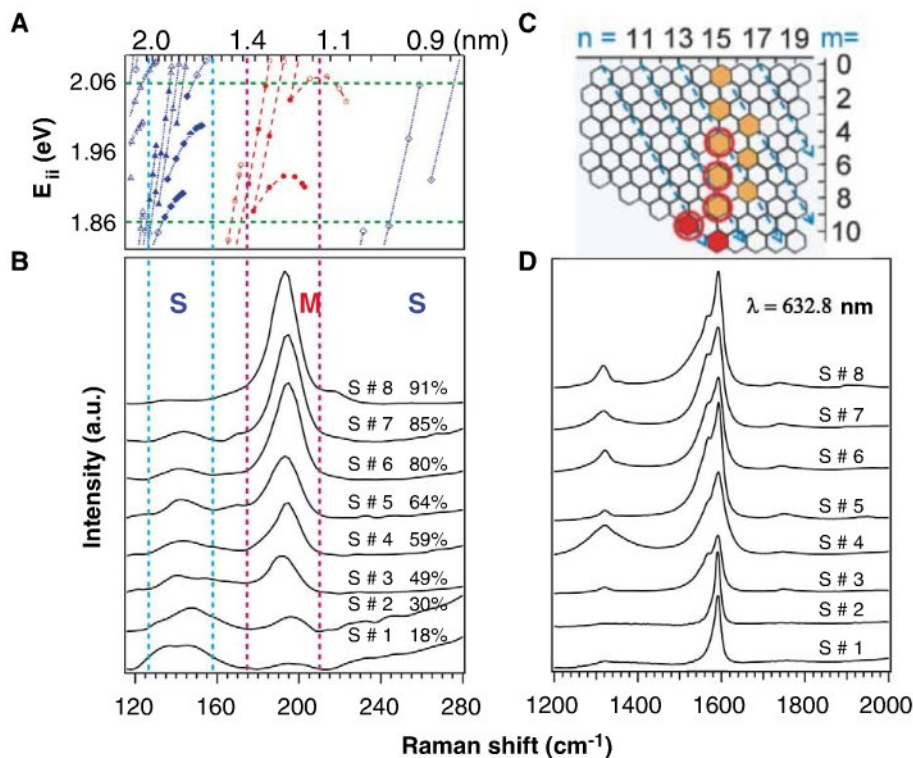


Fig. 1. The Raman spectra ($\lambda = 632.8$ -nm laser wavelength) of SWNTs grown from preliminarily annealed Fe catalysts under different conditions. (A) Populations of the tubes whose optical transition energies E_{ii} are under resonance with laser excitation = 1.96 ± 0.1 eV (solid blue symbols, semiconducting tubes; solid red dots, metallic tubes). (B) Evolution of RBMs for semiconducting and metallic SWNTs, depending on the annealing ambient and duration. The estimated percentage of metallic tubes in each sample is shown. a.u., arbitrary units. (C) Chiral indices (n , m) assignments of the metallic tubes (open red circles, the tubes' chiralities that are matching closely with observed RBM peaks; red solid hexagons, metallic tubes; yellow solid hexagons, semimetallic tubes). (D) Evolution of G-band of corresponding SWNTs samples.

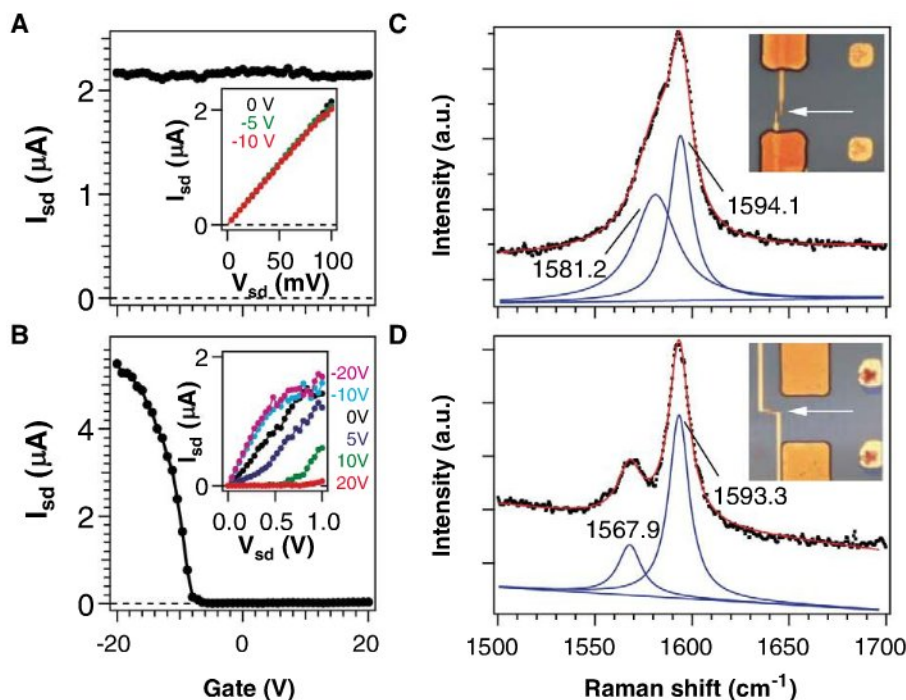


Fig. 2. Simultaneous electrical and Raman measurements ($\lambda = 532$ -nm laser wavelength) of individual SWNTs. Typical electrical behavior for SWNTs incorporated in the FET device assigned as (A) metallic and (B) semiconducting. (Insets) I - V characteristics. V_{sd} , source-drain voltage. Corresponding Raman G-band spectrum for (C) metallic and (D) semiconducting SWNTs. The Lorentzian fittings are shown. (Insets) Optical images of the corresponding devices. The arrows indicate the locations of SWNTs.

nm resonates with roughly equal populations of both metallic and semiconducting tubes for the diameter range of the tubes grown in this study (~ 0.9 to 1.85 nm). Thus, our experiment can provide a reasonably accurate determination of the ratio of metallic tubes to semiconducting tubes (Fig. 1A) (14, 15).

To obtain each spectrum, we averaged 50 individual Raman spectra, measured from different spots of the nanotube sample (the laser beam has a diameter of ~ 1 μm at the sample, and the distance between spots is ~ 10 μm). We observed two distinguishable regions in the RBM spectrum: (i) one in the range from 120 to 160 cm^{-1} , which is assigned to semiconducting tubes (S_{33}), and (ii) another band in the range from 160 to 230 cm^{-1} , which is assigned to metallic tubes (M_{11}). Figure 1B (S#1 and S#2) shows the Raman RBM spectra of the SWNTs grown on the Fe catalyst annealed under Ar:H₂ (9:1) and He:H₂ (9:1) for 5 min at 860°C in the presence of ~ 3.5 mtorr H₂O. The replacement of Ar with He leads to an increase in R from 0.34 to 0.77. Furthermore, with increasing H₂ content (Ar:H₂ at 8:2), the intensity of the RBM peaks originating from the semiconducting tubes declines, whereas the intensity for the RBM of the metallic tubes increases. This results in a variation of the corresponding RBM integral intensity ratio from $R = 0.34$ to 1.71 (S#1 and S#3), with a maximum of $R = 2.64$ in the case of the He:H₂ (8:2) ambient (S#4).

Next, to obtain the highest possible value of R , we performed parametrical studies of R for both He- and Ar-supported environments in the presence of ~ 3.5 mtorr H₂O and for different catalyst annealing durations at 860°C (from <1 min to a maximum of 10 min, S#5 to S#8). We achieved an R value of 3.22 for SWNTs grown from Fe particles annealed in an Ar:H₂ (8:2) ambient for 2 min and a dramatically high R value of 20.2 for He:H₂ (8:2) conditioning ambient for a 1 min annealing duration (S#5 and S#8, respectively). It is straightforward to conclude that for sample S#1, the RBM band of the as-grown tubes is dominated by semiconducting tubes ($R = 0.34$, S#1), whereas in the case of sample S#8, the dominant contribution corresponds to metallic tubes ($R = 20.2$). The G-band spectra (Fig. 1D) display transitions from Lorentzian to Breit-Wigner-Fano line shapes for the corresponding sequence of the samples (13) (fig. S2), and this supports these conductivity assignments (Fig. 1C) (16).

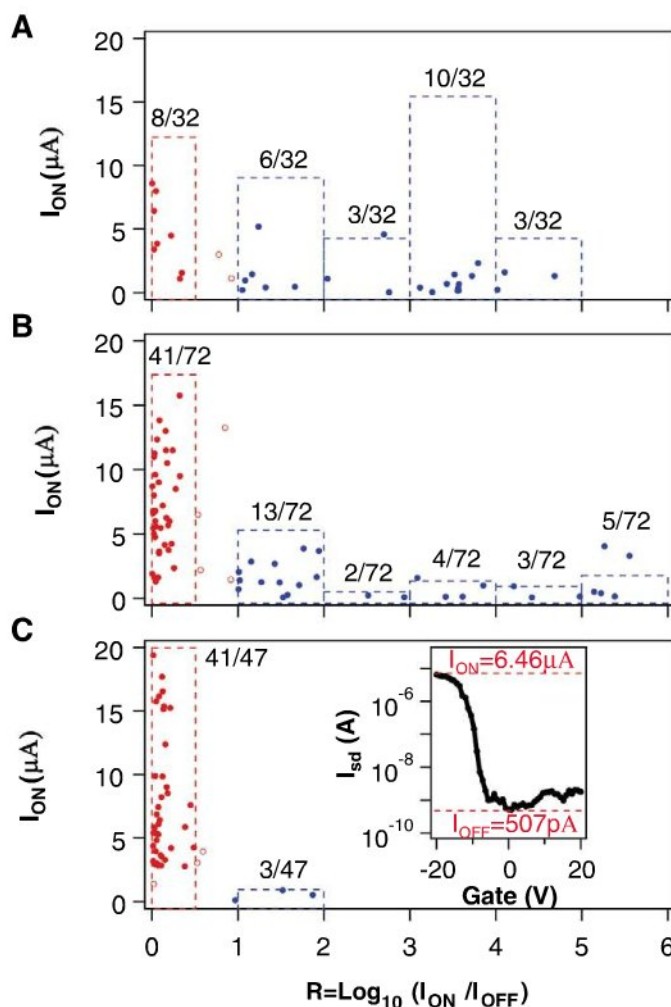
To obtain a reasonable quantitative estimation of the percentage of metallic tubes, we compared the integrated RBM peaks of the Raman spectra with the spectra of a reference sample (2, 11, 12). The use of commercially available high-pressure CO conversion (HiPco) SWNTs as the reference sample [we estimated a 37:63 ratio of metallic tubes to semiconducting tubes on the basis of photoluminescence measurements; see table S1, which contains a value close to that reported in (11) at a 39:61 ratio] results in a determination of

a $\sim 96\%$ metallic tube fraction in the sample with the highest value of R (20.2, S#8). However, HiPco SWNTs show a noticeably different diameter distribution than do our samples, which may cause a large inaccuracy in this estimation, as the optical transitions are sensitive to the tube diameter. Therefore, we prepared reference samples of well-dispersed individual tubes grown on the same silicon substrate under analogous conditions as sample S#1 with $R = 0.34$ (Ar-supported ambient) (Fig. 1B). The substrate had special marks for identification of each tube by atomic force microscopy (AFM) (13) (fig. S3). We used eight laser excitation wavelengths (488, 514, 532, 570, 582, 610, 647, and 675 nm) for assignment of the tube's conductivity, and we made sure that each laser spot interrogated only one tube. We assigned a total of 80 different tubes as metallic or semiconducting based on the approach of previous theoretical and experimental studies (17, 18). Among this population, five tubes could not be assigned. We found that the percentage of metallic tubes in this sample was $18\% (\pm 3\%)$.

To independently verify these Raman spectra analyses, we also performed transport measurements. The assignment of each individual nanotube was based on field-effect transistor

(FET) performance (fig. S4, A and B). Overall, 32 FET devices were prepared and characterized according to their source-drain current (I_{sd}) versus gate voltage (V_g) behavior (Fig. 2, A and B). The statistics of the metallic-versus-semiconducting assignment process (19) are presented in Fig. 3A and show that $25\% (\pm 5\%)$ of the tubes were metallic nanotubes, and the other 75% were semiconducting nanotubes (two tubes were indeterminate), which is in reasonable agreement with the assignments based on Raman spectra results. Figure 2 shows an example of simultaneous electrical (Fig. 2, A and B) and Raman (Fig. 2, C and D) characterizations of the same tube, where the Raman profile also correlates with the electrical performance. Similar studies performed on the individual SWNTs grown on the catalysts annealed under He-supported ambient (~ 3.5 mtorr of H₂O), analogous to the conditions of sample S#4 with $R = 2.64$, show $\sim 50\%$ metallic tubes according to Raman assignments (a total of 119 tubes were assigned) and $\sim 57\% (\pm 5\%)$ based on FET performance (72 FET devices were used for electrical characterizations) (Fig. 3B). These results confirm that the catalyst conditioning ambient affects the relative abundance of metallic and semiconducting tubes.

Fig. 3. Individual carbon nanotube FET device characterizations and histograms of assigned tubes for samples based on (A) S#1, (B) S#4, and (C) S#8. Red dots, metallic behavior; blue dots, semiconducting behavior (circles are not assigned). The inset in (C) shows the notation used for the electrical characteristics. I_{ON} is the on-state current, whereas I_{OFF} is the off-state current.



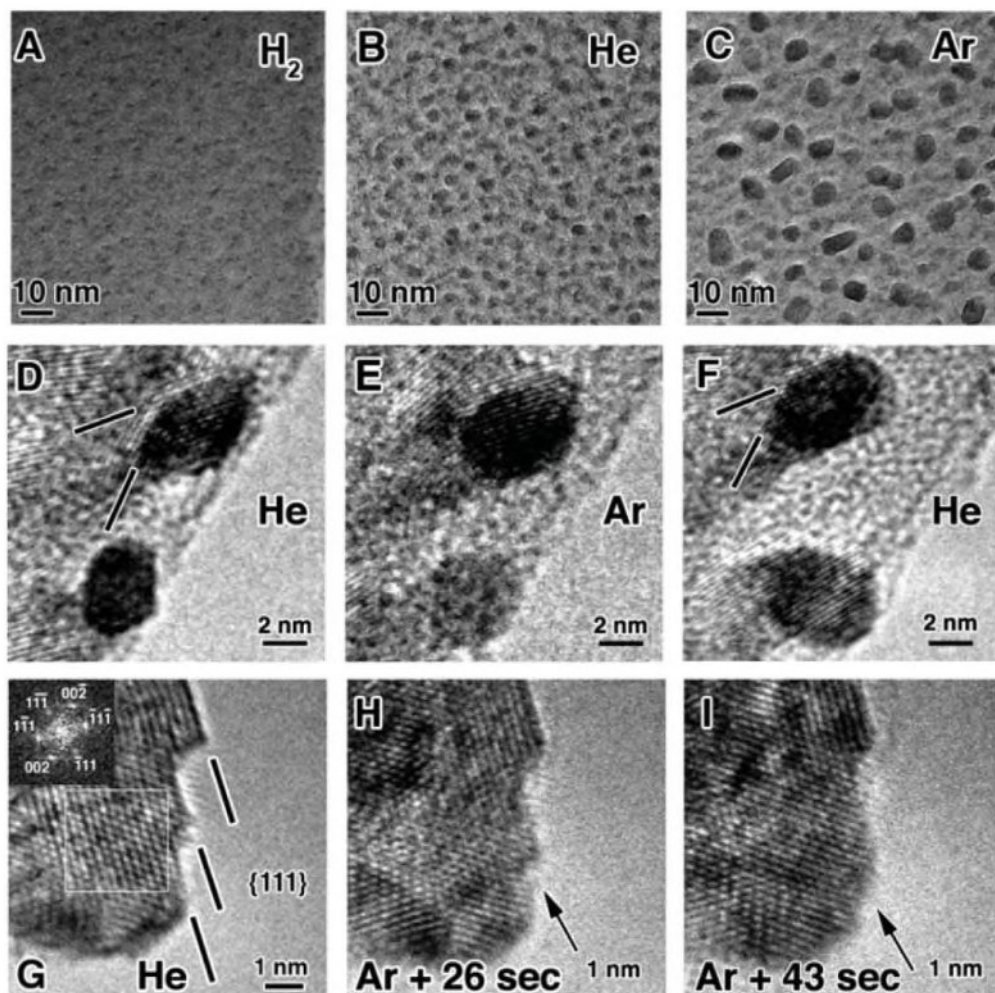
By using the prepared reference sample, we estimated the variation of the metallic tube contribution in the samples from ~18 up to ~91% (Fig. 1, from S#1 to S#8), depending on the ambient used for catalyst conditioning based on Raman spectra. To verify the accuracy of these estimations, independent quantification of the fraction of semiconducting nanotubes was made by comparing the number densities of SWNTs in images from near-infrared photoluminescence (which samples semiconducting species) and AFM (which samples all species) (13). This study indicated the presence of 15% (2.6% SE) semiconducting nanotubes and thus 85% metallic nanotubes for the sample S#6. This is in reasonable agreement with the Raman determinations (20 and 80%, respectively) (13). Moreover, for the sample with the highest estimated metallic content (91%), we performed electrical measurements as well (47 individual tube-based FET devices). These measurements indicated that 87% ($\pm 6\%$) of the nanotubes were metallic (Fig. 3C), thereby supporting the estimated values. Although there were a few samples with percentages of metallic tubes in the range of 87 to 91%, SWNTs with a metallic tube concentration in the range of ~70 to 85% have been grown repeatedly.

To distinguish the effect of H_2 and H_2O species on the metallic-to-semiconducting ratio of the as-grown tubes, we performed a series of experiments using catalyst particles that were in situ annealed in only reductive (H_2) ambient supported by Ar or He without the presence of H_2O ($<7.6 \times 10^{-4}$ mtorr). In these cases, we did not observe any difference between the use of Ar- or He-supported ambient (fig. S5). Thus, the presence of H_2 alone in the ambient does not lead to the preferential growth of metallic tubes. One can therefore conclude that it is the presence of H_2O in the catalyst annealing ambient supported by He that promotes the growth of metallic tubes, whereas the same amount of H_2O supported by Ar ambient is more favorable for semiconducting tubes. These facts also rule out the possibility of in situ modification of the electronic structure of the tubes via chemisorption of ambient species (13, 20).

To gain insight into the mechanism by which the catalyst conditioning could alter the ratio of metallic tubes-to-semiconducting tubes, we performed in situ transmission electron microscopy (TEM) observations of the SiO_2 -supported Fe nanocatalysts in H_2O , H_2/H_2O , Ar/ H_2O , and He/ H_2O gaseous environments under pressures of H_2O $\sim 10^{-2}$ and 500 mtorr for H_2 , He, and Ar,

respectively. We used the same catalyst preparation procedure as for the ex situ growth of nanotubes (13). Before TEM measurements, we carried out in situ reduction of the particles by exposing them to hydrogen gas at 500°C at a pressure of 500 mtorr for 3 hours. The TEM images reveal a strong difference in the ripening behavior of Fe nanoparticles depending on the gas environment at 500°C (Fig. 4, A to C). Under H_2/H_2O ambient, we observed a negligible change in particle size, even after 90-min exposure. However, after 60 min in He/ H_2O ambient, there is a small but noticeable increase in the particles' size, whereas a dramatic ripening is observed after only 15 min of annealing in the Ar/ H_2O environment. This ripening behavior under Ar/ H_2O is notable and becomes even more severe as the Ar gas pressure increases up to ~1.3 torr (fig. S6). Remarkably, high-resolution images taken at 500°C upon introducing He/ H_2O and Ar/ H_2O gas reveal that the particles also undergo reversible shape changes (Fig. 4). In the presence of a He/ H_2O ambient (Fig. 4D), the particle is very faceted, with sharp corners, similar to our observations in H_2/H_2O ambient. In contrast, upon switching to Ar/ H_2O ambient, the particle becomes more rounded (Fig. 4E). Upon returning to He/ H_2O ,

Fig. 4. High-resolution transmission electron micrographs of Fe catalyst as a function of gas environment. (A to C) Size evolution of Fe catalysts after 60 min under H_2 (A), He (B), and Ar (C) at 500°C and 500 mtorr. (D to F) Series of images from the same two Fe catalyst particles held at 500°C, as the gas overpressure is changed from (D) 500 mtorr He to (E) 500 mtorr Ar to (F) 500 mtorr He. (G to I) Series of images from a larger Fe catalyst particle along a 110 zone axis. (G) Image taken in 500 mtorr He at 500°C, showing very strong {111} facets. The inset diffractogram confirms the zone axis orientation. (H) After the introduction of Ar, local degradation of the facets begins. (I) With further time at 500°C in the Ar environment, the facet has been completely removed. For all cases, the H_2O with base pressure of 10^{-2} mtorr is present. Arrows in (H) and (I) indicate the gradual defaceting features over time.



the same strong faceting is yet again observed (Fig. 4F) (movie S1). This behavior was found to be ubiquitous, with all particles in He showing stronger faceting, and those in Ar exhibiting a more rounded shape.

We also investigated relatively larger catalyst sizes to more clearly observe the evolution of catalyst shapes. Figure 4G indicates that the equilibrium shape of the catalyst in the 500°C and 500 mtorr of He/H₂O environment has {111} facets with very sharp edges, which is exactly the same observation as in Fig. 4D. Figure 4, H and I, (movie S2) tracks the gradual defaceting of the hill-and-valley structure with increasing time at the same temperature after the removal of He/H₂O and the addition of Ar/H₂O. All of the observations reflected in Fig. 4 are consistent: Catalysts in the He/H₂O environment are strongly faceted. Additionally, the fact that the particles are more faceted in H₂/H₂O and He/H₂O ambients is consistent with the observation of a reduced ripening rate.

Broadly, the adsorbent-induced variation of the surface free energy $\Delta\gamma$ of the facets with Miller indices (h, k, l) on the particle can be expressed by

$$\Delta\gamma_{h,k,l} = \Delta\gamma_{h,k,l}(P, T, C_s, \theta_o, k_d, S) \quad (1)$$

where P is the partial pressure of the adsorbate, T is the temperature, C_s is the surface concentration of the particle surface atoms, θ_o is the saturation coverage of the adsorbate, k_d is the desorption rate constant, and S is the sticking probability of the adsorbate (21, 22). Our TEM data suggest that the surface energy anisotropy of specific facets of Fe is affected differently by the He/H₂O and Ar/H₂O ambients, resulting in the observed dynamic alterations in the particle shape. It is unlikely that the inert gas itself causes a difference in surface free energy via adsorption at high temperatures. Therefore, the entirety of our data set indicates that it is most probable that H₂O adsorption is responsible for the induced surface reconstruction (23). The adsorption pathway can be either dissociative (with formation of adsorbed hydroxyl, atomic oxygen, and atomic hydrogen species on the surface) or molecular (24). However, analysis of the catalyst structure (through the use of diffractograms produced by fast Fourier transforms of the images, shown as an inset to Fig. 4G, indicating that the particles are γ -Fe) and observation of reversible shape changes (Fig. 4, D to F) suggest that there is no strong oxygen binding, which could have an impact on tube growth (25, 26). The TEM measurements were performed under conditions of dynamic adsorption/desorption equilibrium with both H₂O and either He or Ar gas present. Both the observation of an adsorbent-induced reversible shape reconstruction and severe particle ripening under the Ar/H₂O environment (fig. S6) are consistent with the adatom's coverage of the Fe particles being greater in the He versus the Ar ambient [$\theta_{(He+H_2O)} > \theta_{(Ar+H_2O)}$]. This would also be correlated to the resulting,

relatively low density of grown tubes under Ar-supported ambient (fig. S1). Thus, it appears that the addition of H₂O not only assists the super growth of nanotubes by etching the amorphous carbon (27) and controls the particle diameter by retarding the effect of Ostwald ripening (28), but also alters the shape of the particle. Remarkably, Yamada *et al.* (29) observed the alteration of carbon coated Fe catalysts into flatter particles upon the removal of the carbon coating by water treatment, under standard water-assisted nanotube growth conditions. There are other adsorbates (such as CO and O₂) that, in combination with the carbon source, may be even more effective at altering the catalyst size and morphology. Our results indicate that, with further optimization, direct control over nanotube structure during growth may well be feasible (30, 31).

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- We thank M. S. Dresselhaus and J. Kong for help in Raman measurements, B. Weisman and A. Naumov for photoluminescence measurements, B. I. Yakobson for discussions, and B. Maruyama for assistance. A patent related to this work has been submitted (U.S. patent application no. 12/511,047). This research was supported by the Honda Research Institute USA.

Supporting Online Material

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Materials and Methods

Figs. S1 to S6

Table S1

References

Movies S1 and S2

11 June 2009; accepted 6 August 2009

10.1126/science.1177599

Chiral Organic Ion Pair Catalysts Assembled Through a Hydrogen-Bonding Network

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Research to develop structurally discrete, chiral supramolecular catalysts for asymmetric organic transformations has met with limited success. Here, we report that a chiral tetraaminophosphonium cation, two phenols, and a phenoxide anion appear to self-assemble into a catalytically active supramolecular architecture through intermolecular hydrogen bonding. The structure of the resulting molecular assembly was determined in the solid state by means of x-ray diffraction analysis. Furthermore, in solution the complex promotes a highly stereoselective conjugate addition of acyl anion equivalents to α,β -unsaturated ester surrogates with a broad substrate scope. All structural components of the catalyst cooperatively participate in the stereocontrolling event.

Nature harnesses weak interactions, particularly hydrogen bonds, to construct biologically active supramolecular architectures, as demonstrated by the three-dimensional structures of enzymes and nucleic acids. Inspired by these biological systems, research for the development and application of supramolecular catalysts assembled through noncovalent interactions has attracted interest in the fields of both selective chemical synthesis and molec-

ular recognition (1). Despite important advances in the elaboration of self-assembled molecular receptors and catalyst systems, however, rational design of chiral supramolecular catalysts for stereoselective bond-forming reactions by the use

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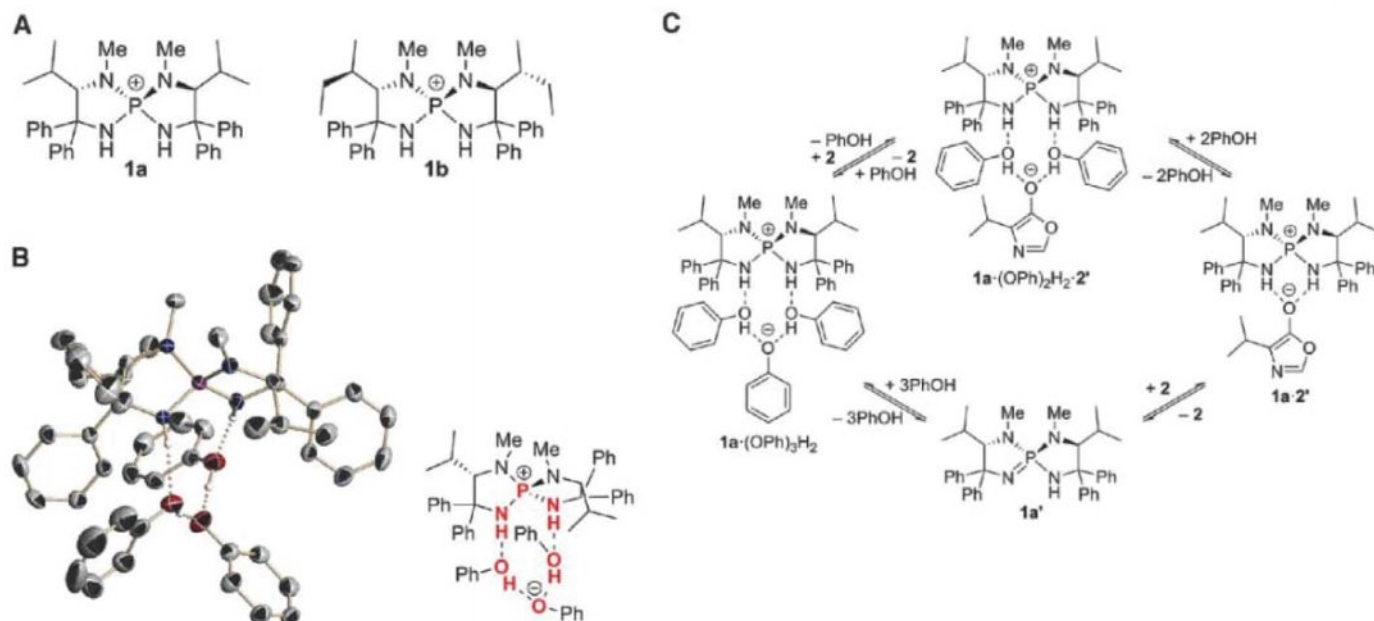
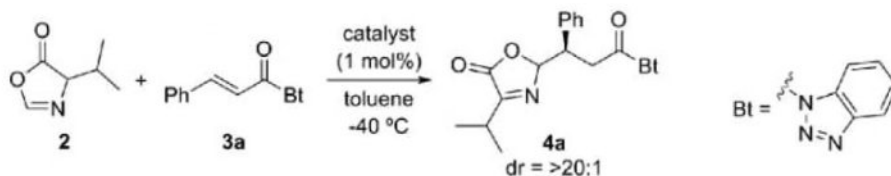


Fig. 1. (A) Structures of chiral tetraaminophosphonium cations. (B) Oak Ridge thermal ellipsoid plot diagram of **1a**·(OPh)₃H₂. All calculated hydrogens were omitted for clarity. (C) Plausible modes of assembly in catalyst and enolate.

of discrete molecular associations remains a great challenge (1–5). Described here are systems in which chiral and achiral small molecules spontaneously assemble through a well-defined array of intermolecular hydrogen bonds to produce structured chiral organic ion pairs. The distinctive feature of the supramolecular ion pair assembly as a chiral molecular catalyst is that its stereocontrolling ability can be fine-tuned by structural modification of both chiral and achiral components. We developed a catalyst for the highly enantioselective conjugate addition of acyl anion equivalents to α,β -unsaturated ester surrogates.

We initially prepared chiral tetraaminophosphonium phenoxides as part of our broader exploration of chiral organic ion pairs for cooperative asymmetric catalysis (6). The requisite cation framework **1a** (Fig. 1A) was readily synthesized from the parent α -amino acid, L-valine, as its chloride salt (7). Then, the anion was exchanged with hydroxide, and subsequent neutralization with phenol (8) yielded **1a**·(OPh)₃H₂. The solid-state structure of **1a**·(OPh)₃H₂ was unambiguously determined by means of single-crystal x-ray diffraction analysis (Fig. 1B). Surprisingly, the analysis revealed a molecular assembly in which an aminophosphonium cation, two phenols, and a phenoxide anion were aligned through a 10-membered cyclic network of intermolecular hydrogen-bonding interactions. The hydrogen-bonding donor capability of the phenols seems to be enhanced by an additional hydrogen bond from the N–H protons of the phosphonium cation, thus facilitating the construction of an antidromic circular network (9–11). Another salient feature of the assembly was that the stereochemical information in the chiral cation moiety was effectively relayed by the two phenol molecules, extending

Table 1. Effect of each component of the catalyst assembly. Unless otherwise noted, the reactions were performed with 0.22 mmol of **2** and 0.2 mmol of **3a** in the presence of catalyst (1 mol %) in 2.0 mL of toluene at -40°C under argon atmosphere. The catalyst concentrations are indicated. The isolated yields are reported. All diastereomeric ratios (dr) and ee were determined by means of ^1H nuclear magnetic resonance (NMR) (500 MHz) analysis of crude aliquots and chiral stationary phase high-performance liquid chromatography (HPLC), respectively. The relative stereochemistry was not determined.



Entry	Catalyst	Conc (mM)	Time (h)	Yield (%)	ee (%)
1	1a ·(PhO) ₃ H ₂	1	6	99	60
2	1a *	1	2	99	34
3	1a * + 3PhOH	1	10	98	62
4	1a ·2' + 3PhOH	1	16	87	61
5	1a ·(4-Me-C ₆ H ₄ O) ₃ H ₂	1	4	96	58
6	1a ·(4-Cl-C ₆ H ₄ O) ₃ H ₂	1	10	97	75
7	1a ·(2-Cl-C ₆ H ₄ O) ₃ H ₂	1	12	94	63
8	1a ·(3-Cl-C ₆ H ₄ O) ₃ H ₂	1	6	93	70
9	1a ·(3,5-Cl ₂ -C ₆ H ₃ O) ₃ H ₂	1	16	92	80
10*	1a ·(3,5-Cl ₂ -C ₆ H ₃ O) ₃ H ₂	2	24	99	85
11†	1a ·(3,5-Cl ₂ -C ₆ H ₃ O) ₃ H ₂	5	18	98	89
12‡	1a ·(3,5-Cl ₂ -C ₆ H ₃ O) ₃ H ₂	10	20	94	89
13§	1a ·(3,5-Cl ₂ -C ₆ H ₃ O) ₃ H ₂	10	4	99	87
14§	1b ·(3,5-Cl ₂ -C ₆ H ₃ O) ₃ H ₂	10	4	95	95

*Two mol % of catalyst was used.
of toluene was used as solvent.

†Five mol % of catalyst was used.

‡Ten mol % of catalyst was used.

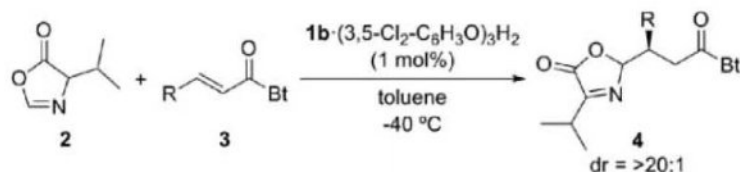
§0.2 mL

the chiral environment around the remotely located phenoxide anion.

On the basis of these serendipitous observations, we explored the use of **1**·(OAr)₃H₂ as a chiral molecular catalyst for synthetically valuable stereoselective transformations. For this pur-

pose, we chose 2-unsubstituted oxazol-5(4H)-one, namely azlactone, **2** (Fig. 1C and Table 1) as a pro-C1-nucleophile (12) with the expectation that the enolate **2'** generated through deprotonation by **1**·(OAr)₃H₂ would behave as a suitable phenoxide anion equivalent; as such, it would

Table 2. Scope of α,β -unsaturated acylbenzotriazole **3**. The reactions were performed with 0.22 mmol of **2** and 0.2 mmol of **3** under the influence of **1b**·(3,5-Cl₂-C₆H₃O)₃H₂ (1 mol %) in 0.2 mL of toluene at -40°C under argon atmosphere. The isolated yields are reported. All dr and ee were determined by means of ¹H NMR (500 MHz) analysis of crude aliquots and chiral stationary phase HPLC, respectively.



Entry	R (3)	Time (h)	Yield (%)	ee (%)	Prod
1	4-MeO-C ₆ H ₄ (3b)	24	98	97	4b
2	4-Br-C ₆ H ₄ (3c)	21	98	98	4c
3	2-Me-C ₆ H ₄ (3d)	8	90	93	4d
4	3-Br-C ₆ H ₄ (3e)	4	96	95	4e
5	1-Naph (3f)	12	91	95	4f
6	2-Furyl (3g)	22	91	96	4g
7	Me (3h)	2	97	96	4h *
8	Me(CH ₂) ₄ (3i)	1	96	95	4i
9	Ph(CH ₂) ₂ (3j)	2	92	96	4j
10	Cyclohexyl (3k)	4	93	98	4k

*Absolute configuration of the product **4h** was determined, after conversion to the known compound **6** (Fig. 2), by comparison of the optical rotation with the literature value (7).

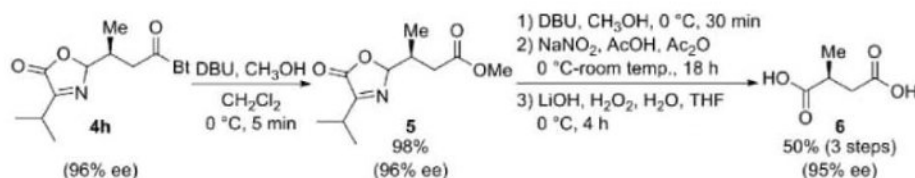


Fig. 2. Conversion of **4h** to optically active methylsuccinic acid **6**. DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; Ac, acetyl; THF, tetrahydrofuran.

be captured into a similar hydrogen-bonding network without negatively affecting the preorganization [**1a**·(OPh)₂H₂·**2'**] (Fig. 1C). If this held true, the structures of not only the chiral aminophosphonium cations but also the achiral phenols would affect the stereo-determining step of the addition reaction of the enolate **2'**. To verify this hypothetical synergistic effect of the structural components of the supramolecularly assembled ion-pair catalyst, we examined the conjugate addition of **2** to α,β -unsaturated acylbenzotriazole **3** (**13**), a reactive and readily derivatizable Michael acceptor, under the influence of **1**·(OAr)₃H₂.

The initial attempt entailed stirring a mixture of **2**, **3a**, and **1a**·(OPh)₃H₂ in toluene at -40°C, giving rise to essentially diastereomerically pure adduct **4a** in a quantitative yield with 60% enantiomeric excess (ee) (Table 1, entry 1). In contrast, use of the independently prepared conjugate base of **1a** (triaminoiminophosphorane **1a'**) (Fig. 1C) (**14**) as a catalyst led to the formation of **4a** with 34% ee (entry 2). These results clearly indicate the importance of the phenolic component of **1a**·(OPh)₃H₂ in enhancing enantioselectivity. Whereas the reaction with **1a'** proceeds

through the generation of chiral aminophosphonium enolate **1a**·**2'**, the expected, highly organized **1a**·(OPh)₂H₂·**2'** complex could be involved in the **1a**·(OPh)₃H₂-catalyzed system, and the two phenols may play a key role in creating an attractive chiral environment around the azlactone enolate **2'**.

Treatment of iminophosphorane **1a'** with phenol (3 equiv), followed by the sequential addition of **3a** and **2**, resulted in the formation of **4a** in 98% yield with 62% ee after 10 hours of stirring (entry 3). The reactivity and selectivity were scarcely affected by the order of the addition of phenol and azlactone **2** (entry 4). These results suggest that the preorganization of the catalyst is not a prerequisite for the generation of **1a**·(OPh)₂H₂·**2'**, which could be self-assembled from **1a'**, phenols, and **2** by way of either **1a**·(OPh)₃H₂ or **1a**·**2'**. These observations implied that the selectivity could be tunable through structural modification of the achiral phenolic component. Indeed, although the selectivity decreased when phenols of **1a**·(OPh)₃H₂ were replaced by 4-methylphenol, a series of chloro-substituted phenols, particularly 3,5-

dichlorophenol, induced substantial increases in enantioselectivity (entries 5 to 9) (table S1 and SOM text). Furthermore, we confirmed the effect of the catalyst concentration on the propensity for molecular association in solution. As we had assumed, both increased catalyst loading and decreased solvent volume improved enantioselectivity (entries 10 to 12 and 13), which also argues for the role of a requisite molecular assembly (fig. S1 and SOM text). Structural modification of the chiral cationic moiety by using L-isoleucine-derived **1b** (see Fig. 1A) also appeared to be crucial for achieving the highest selectivity (entry 14). Consequently, all the structural components of the self-assembled catalyst cooperatively participate in realizing the rigorous stereocontrol.

With the optimal catalyst structure and reaction conditions in hand, we conducted further experiments to probe the scope of α,β -unsaturated acylbenzotriazole **3**, and the representative results are summarized in Table 2. Generally, use of 1 mole percent (mol %) of **1b**·(3,5-Cl₂-C₆H₃O)₃H₂ and 1.1 equivalents of **2** was sufficient for a smooth reaction, giving **4** in high yield with excellent enantioselectivity. With aryl-substituted **3**, this method tolerated the incorporation of both electron-withdrawing and electron-donating substituents at an arbitrary position on the aromatic scaffold (entries 1 to 4). In addition, fused- and heteroaromatic β -substituents had no influence on the stereochemical outcome (entries 5 and 6). A range of primary and secondary alkyl groups were also nicely accommodated (entries 7 to 10).

The acylbenzotriazole and azlactone moieties of product **4** can be selectively converted to various useful functional groups, thus highlighting the synthetic potential of the present highly stereoselective conjugate addition protocol. For example, **4h** (96% ee) was successfully derivatized into optically active methylsuccinic acid (**6**) in four steps without loss of the enantiomeric excess (Fig. 2).

We believe these results encourage further research efforts to determine the minimal chiral or achiral structural bias necessary to induce spontaneous yet discrete associations between simple organic molecules through weak noncovalent interactions. The resultant catalytically active and selective structures may greatly expand the possibilities for designing functional supramolecular catalysts.

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15. Financial support was provided by a Grant-in-Aid for Scientific Research on Priority Areas "Chemistry of

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1176758
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Tables S1 to S3
References

26 May 2009; accepted 18 August 2009
Published online 27 August 2009;
10.1126/science.1176758
Include this information when citing this paper.

Nitrous Oxide (N₂O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century

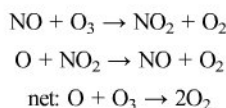
A. R. Ravishankara,* John S. Daniel, Robert W. Portmann

By comparing the ozone depletion potential-weighted anthropogenic emissions of N₂O with those of other ozone-depleting substances, we show that N₂O emission currently is the single most important ozone-depleting emission and is expected to remain the largest throughout the 21st century. N₂O is unregulated by the Montreal Protocol. Limiting future N₂O emissions would enhance the recovery of the ozone layer from its depleted state and would also reduce the anthropogenic forcing of the climate system, representing a win-win for both ozone and climate.

The depletion of the stratospheric ozone layer by human-made chemicals, referred to as ozone-depleting substances (ODSs), was one of the major environmental issues of the 20th century. The Montreal Protocol on Substances That Deplete the Ozone Layer (1), MP, emerged from the Vienna Convention for the Protection of the Ozone Layer (2). The MP has been highly successful in reducing the emissions, growth rates, and concentrations of chlorine- and bromine-containing halocarbons, the historically dominant ODSs (3), and has limited ozone depletion and initiated the recovery of the ozone layer.

The relative contributions of various ODSs to ozone layer depletion are often quantified by the ozone depletion potential (ODP) (4). An ODP relates the amount of stratospheric ozone destroyed by the release of a unit mass of a chemical at Earth's surface to the amount destroyed by the release of a unit mass of chlorofluorocarbon 11, CFC-11 (CFCl₃). ODPs are widely used for policy formulation because of their simplicity in quantifying the relative ozone-destroying capabilities of compounds.

Through the work of Crutzen (5) and Johnston (6), nitrogen oxides (NO_x = NO + NO₂) are also known to catalytically destroy ozone via



The primary source of stratospheric NO_x is surface N₂O emissions [(7) and references therein]. N₂O has been thought of as primarily a natural atmospheric constituent, but the influence of its changes on long-term changes in ozone concentrations has also been examined (8–10).

Nitrous oxide shares many similarities with the CFCs, historically the dominant ODSs. The CFCs and N₂O are very stable in the troposphere, where they are emitted, and are transported to the stratosphere where they release active chemicals that destroy stratospheric ozone through chlorine- or nitrogen oxide-catalyzed processes. They both have substantial anthropogenic sources. Unlike CFCs, N₂O also has natural sources, akin to methyl bromide, which is another important ODS. Assigning an ODP for N₂O and separating out the natural and anthropogenic emissions are therefore no more conceptually difficult than they are for methyl bromide.

In spite of these similarities between N₂O and previously recognized ODSs and in spite of the recognition of the impact of N₂O on stratospheric ozone, N₂O has not been considered to be an ODS in the same sense as chlorine- and bromine-containing source gases. The signatories to the Vienna Convention (2) have agreed in Article 2 (General Obligations) to "Adopt appropriate legislative or administrative measures ... to control, limit, reduce or prevent human activities under their jurisdiction or control should it be found that these activities have or are likely to have adverse effects resulting from modification or likely modification of the ozone layer." Yet N₂O remains unregulated by the MP (1).

Here, we present the ODP of N₂O to be positive and nonzero and show that N₂O is an ozone-

depleting substance on the basis of the extent of ozone depletion it causes. Indeed, current anthropogenic ODP-weighted N₂O emissions are the largest of all the ODSs and are projected to remain the largest for the rest of the 21st century.

We have calculated the ODP of N₂O by using the Garcia and Solomon two-dimensional (2D) model [(11) and references therein], which is similar to models used previously for such calculations (12, 13). The ODP of N₂O under current atmospheric conditions is computed to be 0.017. This value is comparable to the ODPs of many hydrochlorofluorocarbons (HCFCs) (3) such as HCFC-123 (0.02), -124 (0.022), -225ca (0.025), and -225cb (0.033) that are currently being phased out under the MP. We conclude that the value of the ODP of N₂O is robust because (i) our similarly calculated ODPs for CFC-12 (1.03) and HCFC-22 (0.06) agree with the accepted values (3); (ii) ozone depletion by NO_x from N₂O dominates the chemical control of ozone in the mid-stratosphere (13), a region well represented with 2D models; and (iii) ozone reductions by enhanced N₂O have been reported in other studies (8, 10, 14), although no published study, to the best of our knowledge, has previously presented an ODP for N₂O.

We examine here a few important factors that influence the ODP of N₂O. At mid-latitudes, chlorine-catalyzed ozone destruction contributes most to depletion in the lowest and upper stratospheres, that is, below and above the ozone maximum. Nitrogen oxides contribute most to ozone depletion just above where ozone concentrations are the largest. This leads to efficient ozone destruction from NO_x (13). The ODP of N₂O is lower than that of CFCs primarily because only ~10% of N₂O is converted to NO_x, whereas the CFCs potentially contribute all their chlorine.

There are important interconnections between the roles of nitrogen oxides with chlorine such that the N₂O ODP may be different from the calculated value in the past and future. It is well known that nitrogen oxides dampen the effect of chlorine-catalyzed ozone destruction via the formation of ClONO₂, which ties up some of the chlorine in a benign form. However, as shown by Kinnison *et al.* (9), other reactions, such as the conversion of ClO to Cl by NO, can offset the damping.

We quantify the dependence of the ODP of N₂O on atmospheric concentrations of chlorine by calculating it for 1959 concentrations of strato-

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spheric Cl_y (essentially preindustrial). We find the ODP for 1959 to be 0.026, showing that Cl_y concentrations have a moderate effect on the efficiency of N_2O -caused ozone destruction. These results for the 1959 and 2000 Cl_y concentrations bracket the range expected for the rest of the 21st century; it shows that the N_2O 's ozone destructiveness per emitted unit mass should increase by about 50% when the stratospheric chlorine loading returns to preindustrial concentrations.

Nitrogen oxide chemistry is also dependent on odd hydrogen, bromine, and methane levels, but the dependence of N_2O 's ODP on these factors is expected to be much smaller than the effect of chlorine (13).

Whereas enhanced stratospheric sulfate aerosols after volcanic injections increase the effectiveness of chlorine to destroy ozone, they will decrease the effectiveness of NO_x emissions by sequestering the catalytically active NO_x in HNO_3 . Such an influence has been observed after the Mount Pinatubo eruption (15). Therefore, we anticipate that the ODP of N_2O will be reduced when the sulfate loading is enhanced. However, high volcanic sulfate loadings are unpredictable and sporadic, and their effects are short-lived, lasting only a few years. We assess the extent of their influence by calculating ODPs at peak sulfate loadings observed after the eruption of Mount Pinatubo (13, 16).

For the remaining discussion, we will use an ODP of 0.017 as though it were independent of atmospheric conditions, atmospheric composition, and time. This value is a conservative choice for the reasons discussed above.

It is important to note that the ODP alone cannot fully quantify the impact of a chemical that is released into the atmosphere. The entire emission history, and even the potential future emission projections, must be considered by using an extensive quantity like ODP-weighted emission as a metric rather than an intensive quantity such as ODP, which only considers the ozone depletion per unit mass. Figure 1 compares the anthropogenic N_2O emissions with those from the major ODSs (now controlled under the MP) for 1987 and 2008. It is clear that ODP-weighted anthropogenic emissions of N_2O were a substantial fraction of the ODP-weighted emissions of CFC-11, CFC-12, and CFC-113 even in 1987, just before the adoption of the MP. They were likely larger than the sum of the ODP-weighted emission of halons and were much larger than that of methyl bromide.

Even though N_2O 's ODP is only 0.017, roughly one-sixtieth of CFC-11s, the large anthropogenic emissions of N_2O more than make up for its small ODP, making anthropogenic N_2O emissions the single most important of the anthropogenic ODS emissions today (Fig. 1). For example, the global anthropogenic emission of N_2O now (produced mainly as a byproduct of fertilization, fossil fuel combustion and industrial processes, biomass and biofuel burning, and a few other processes) is roughly 10 million metric tons per year

compared with slightly more than a million metric tons from all CFCs at the peak of their emissions.

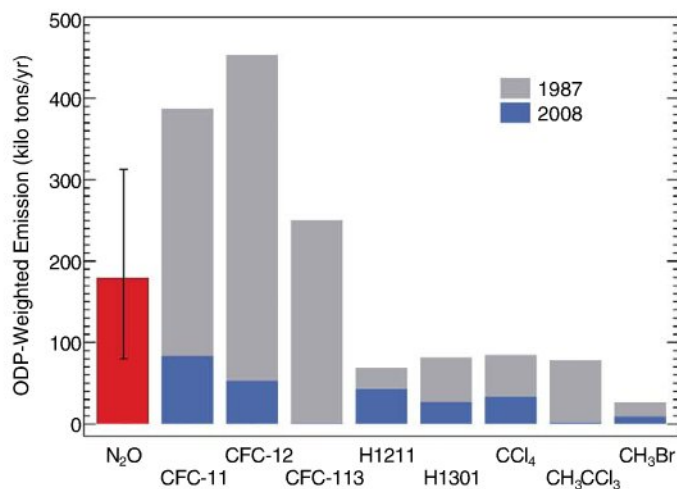
Figure 2 compares estimated ODP-weighted emissions of various ODSs controlled by the MP during the late 20th and all of the 21st centuries [see (13) for details of the calculation]. Recent estimates of expected future N_2O emissions under various greenhouse gas mitigation requirements continue to show that N_2O emissions are unlikely to be lower than they are today, even under the most stringent reduction requirements (17). From the top graph of Fig. 2, it is clear that N_2O is the largest ODS emission today and indeed is expected to remain the largest throughout the rest of this century for all of these emission scenarios. If anthropogenic N_2O emissions were to continue unabated, by 2050 they could represent an ODP-weighted emission in excess of 30% of the peak CFC ODP-weighted emissions of 1987. These fundamental conclusions on the influences of anthropogenic N_2O are not particularly sensitive to the uncertainties in the total anthropogenic emission rate or to the uncertainties in specific sectoral emissions (13).

It should be noted that the largest uncertainty in ODP-weighted emission comparisons comes from the uncertainties in the emission estimates of N_2O , rather than in the calculated ODP. The magnitudes of the sectoral emissions of N_2O , mostly from agricultural practices and industrial sources, are highly uncertain, but the total human-caused emissions are constrained by observed increases in N_2O concentrations and N_2O 's lifetime. The Intergovernmental Panel on Climate Change (IPCC)'s fourth assessment report estimates (18) a total annual emission during the 1990s of 17.7 TgN, of which 6.7 TgN (10.5 million metric tons of N_2O) were anthropogenic in origin.

Nitrous oxide is a greenhouse gas and is controlled under the Kyoto Protocol; it may be controlled via future climate negotiations. Therefore, it is also interesting to compare the contribution of N_2O to climate forcing with the contributions of other major greenhouse gases. The bottom graph of Fig. 2 shows the CO_2 equivalent [100-year global warming potential (GWP) weighted] emissions of various non- CO_2 greenhouse gases. Among these gases, N_2O 's contribution to climate forcing is second only to methane and is already much larger than that of all currently recognized ODSs. These projections of ODP- and GWP-weighted N_2O emissions show that N_2O is an important gas for both the future ozone layer and climate. They also support, and now quantify, previous suggestions that reductions in N_2O emissions would benefit both the ozone layer and climate (10). Numerous N_2O mitigation options are currently available. Examples include more efficient use of fertilizer on cropland (19) and the capture and destruction of byproduct N_2O emissions in chemical processes (e.g., manufacturing adipic and nitric acids) (20). It may be more desirable to reduce nonindustrial N_2O emissions when its ozone layer depletion impact is considered in addition to its impact on climate.

The World Meteorological Organization/United Nations Environment Programme (WMO/UNEP) 2007 assessment (3) states that the largest single option available to hasten ozone layer recovery is the recapture and destruction of ODSs (mostly CFCs and halons) that are already produced but not yet emitted to the atmosphere, that is, the so-called banks. However, much of the banked halocarbons reside in applications that are generally not cost-effective to recover

Fig. 1. Comparison of annual N_2O ODP-weighted emissions from the 1990s [IPCC, 2007 (18, 23)] with emissions of other ozone-depleting substances in 1987, when the emissions of chlorine- and bromine-containing ODSs were near their highest amount, and for 2008. Emissions during 2008 were inferred from observations taken by the Global Monitoring Division, Earth System Research Laboratory, National Oceanic and Atmospheric Administration for CFC-11, CFC-12, Halon 1211 (H1211), Halon 1301 (H1301), and CH_3Br ; all other emissions are taken from WMO (3). ODPs for all, except N_2O , are assumed to be the semi-empirical ODPs from WMO (3). Even at the height of ODS emissions in the 1980s, annual anthropogenic N_2O emissions were the fourth most important. Currently, anthropogenic N_2O emissions represent the largest contribution to ozone-depleting gas emissions. HCFC-22, the most important CFC replacement, would fall below the 1987 amount of CH_3Br for both time periods if included in the figure. The N_2O error bar represents a bottom-up uncertainty range. The lower end of the range is calculated by summing the lowest emissions estimates, and the higher end by summing the highest estimates, of the various individual sources provided by the IPCC (18).



(e.g., foams in buildings) or in applications with continued demand and unavailability of suitable replacements (e.g., halons for fire fighting and CFCs for medical uses). Based on our value of the ODP and the IPCC fourth assessment report emission estimates for N_2O , the total 2005 banks (3) of ODSs are equivalent to roughly 20 years of continued anthropogenic emissions of N_2O at today's rate. Thus, although policy decisions regarding banks of halons and CFCs do represent the largest option for ozone protection today, the effect of N_2O can be expected to dominate in the future as the banks of these ODSs are either released to the atmosphere or are captured and destroyed. Furthermore, the destruction of the existing ODS bank represents a one-time benefit, whereas reductions in N_2O emissions have the ability to continue providing benefits into the future.

We also point out that increases in anthropogenic N_2O emissions or decreases due to abatement strategies would affect a number of issues of importance to stratospheric ozone: (i) it would

affect the date for the recovery of the ozone layer; (ii) it would imply that the use of a single parameter such as equivalent effective stratospheric chlorine (EESC) to estimate the recovery of the ozone layer should be reevaluated; (iii) it would have implications for the recovery of the polar ozone hole that might differ from that of global ozone; (iv) N_2O could be an unintended by-product of enhanced crop growth for biofuel production (21) or iron fertilization to mitigate CO_2 emissions (22). Such an enhancement would lead to the unintended "indirect" consequence of ozone layer depletion and increased climate forcing by an alternative fuel used to curb global warming, as pointed out by Crutzen *et al.* (21).

For historical reasons, it is interesting to compare ozone depletion caused by anthropogenic N_2O emissions with that from the original projections for 500 U.S. supersonic transports (7), SSTs. The total increase in stratospheric NO_x by that fleet of SSTs is comparable to that from today's total anthropogenic N_2O emission, indicative of the significance of anthropogenic N_2O .

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 and S2

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28 May 2009; accepted 12 August 2009

Published online 27 August 2009;

10.1126/science.1176985

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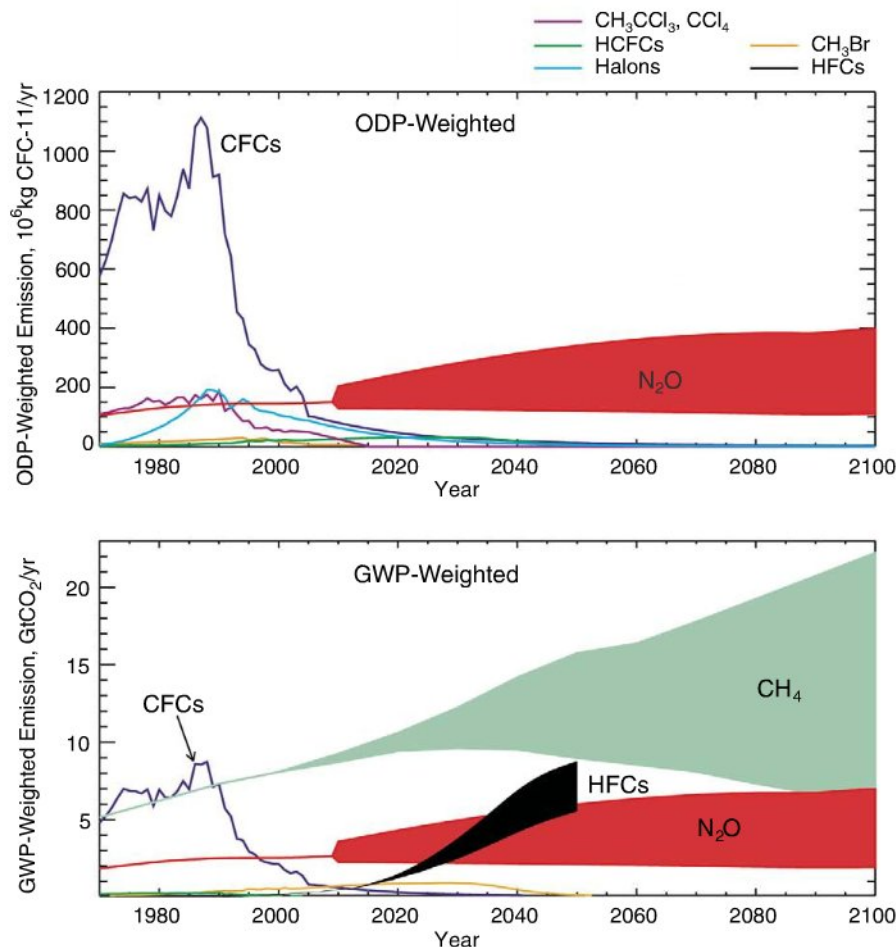


Fig. 2. Historical and projected ODP- and GWP-weighted emissions of the most important ODSs and non- CO_2 greenhouse gases. Non- N_2O ODS emissions are taken from WMO (3). Hydrofluorocarbon (HFC) projections are taken from Velders *et al.* (24), do not include HFC-23, and are estimated assuming unmitigated growth. The HFC band thus represents a likely upper limit for the contribution of HFCs to GWP-weighted emissions. CH_4 emissions represent the range of the Special Report on Emissions Scenarios (SRES) A1B, A1T, A1FI, A2, and B1 scenarios (23). The range of anthropogenic N_2O emissions is inferred from the mixing ratios of these same SRES scenarios [see (13) for details of calculation].

Enhanced Sulfur and Coking Tolerance of a Mixed Ion Conductor for SOFCs: $\text{BaZr}_{0.1}\text{Ce}_{0.7}\text{Y}_{0.2-x}\text{Yb}_x\text{O}_{3-\delta}$

Lei Yang, Shizhong Wang, Kevin Blinn, Mingfei Liu, Ze Liu, Zhe Cheng, Meilin Liu*

The anode materials that have been developed for solid oxide fuel cells (SOFCs) are vulnerable to deactivation by carbon buildup (coking) from hydrocarbon fuels or by sulfur contamination (poisoning). We report on a mixed ion conductor, $\text{BaZr}_{0.1}\text{Ce}_{0.7}\text{Y}_{0.2-x}\text{Yb}_x\text{O}_{3-\delta}$, that allows rapid transport of both protons and oxide ion vacancies. It exhibits high ionic conductivity at relatively low temperatures (500° to 700°C). Its ability to resist deactivation by sulfur and coking appears linked to the mixed conductor's enhanced catalytic activity for sulfur oxidation and hydrocarbon cracking and reforming, as well as enhanced water adsorption capability.

Unlike polymer electrolyte fuel cells, solid oxide fuel cells (SOFCs) can use a wide variety of hydrocarbon fuels (1). Because of their high operating temperatures (600° to 800° C), metal catalysts added to the ceramic anodes can facilitate reforming reactions that generate H_2 and CO from hydrocarbons. The conventional anode for a SOFC, a composite consisting of nickel and yttria-stabilized zirconia (YSZ), has excellent catalytic activity for fuel oxidation, good conductivity for current collection, and unmatched compatibility with YSZ electrolyte for easy cell fabrication, but it is highly susceptible to carbon buildup (coking) and deactivation (poisoning) by contaminants commonly encountered in readily available fuels (2). Some contaminants (e.g., sulfur impurities) can degrade its performance even at parts per million (ppm) levels (3). Sulfur ad-

sorbs strongly on Ni surface and thus blocks the active sites for electrochemical oxidation of fuel, resulting in considerably increased anodic polarization and energy loss.

To overcome these problems, substantial effort has been devoted to the development of new anode materials and novel electrode structures (4–11). For example, the use of ceria-based anodes demonstrated the potential for direct use of methane in a SOFC (4). Later, the use of a composite anode consisting of copper and ceria led to successful operation of a SOFC with higher hydrocarbons than methane, which are more prone to coking because of their higher carbon content (5). However, some practical issues still remain: The low melting point of Cu makes it difficult to fabricate anode-supported cells using conventional co-firing ceramic methods, and the poor catalytic activity of Cu for fuel oxidation limits cell power output. In another approach, a catalyst layer (e.g., Ru-ceria) was applied to a conventional Ni-YSZ anode to allow internal reforming of hydrocarbons. The ef-

fectiveness of this cell structure was confirmed for direct use of iso-octane without coking in a SOFC with power densities of 0.3 to 0.6 W/cm^2 at 670° to 770°C (7). Although this cell design has demonstrated the possibility of a simple low-cost SOFC system with common automotive fuels, the drawbacks include decreased power density, difficulty in current collection, and the high cost of Ru.

Nickel-free conducting metal oxides have also been developed as anode materials, including $\text{La}_{0.75}\text{Sr}_{0.25}\text{Cr}_{0.5}\text{Mn}_{0.5}\text{O}_{3-\delta}$ (with a $\text{Ce}_{0.8}\text{Gd}_{0.2}\text{O}_{2-\delta}$ interlayer) (6), $\text{Sr}_2\text{Mg}_{1-x}\text{Mn}_x\text{MoO}_{6-\delta}$ ($x = 0$ to 1) (9), and doped $(\text{La},\text{Sr})(\text{Ti})\text{O}_3$ (8, 11). These anode materials showed different degrees of improved tolerance to coking, reoxidation, and/or sulfur poisoning under various SOFC operating conditions. In many cases, however, the power densities of the SOFCs using Ni-free oxide anodes are less than those demonstrated by conventional Ni-YSZ-supported SOFCs with thin electrolytes (by more than 50%). This low efficiency arises from difficulties in fabricating thin-YSZ electrolyte on porous oxide anode supports that arise from delamination or formation of undesirable phases. In some cases, inadequate lateral conductivity (or substantial sheet resistance) of Ni-free oxide anodes also contributes to low power density, especially for SOFC designs with long current collection paths, as in fuel cell stacks.

To compensate for these issues, conducting oxides (e.g., $\text{Sr}_{0.8}\text{La}_{0.2}\text{TiO}_3$) have been used as anode-side supports together with an interlayer (e.g., a ceramic-metal composite, or cermet, of Ni and doped ceria) to allow fabrication of an anode-supported thin-electrolyte SOFC via conventional ceramic processing methods (12). This cell design leads to high power densities, much-improved stability against coking in natural gas, and enhanced tolerance to H_2S poisoning.

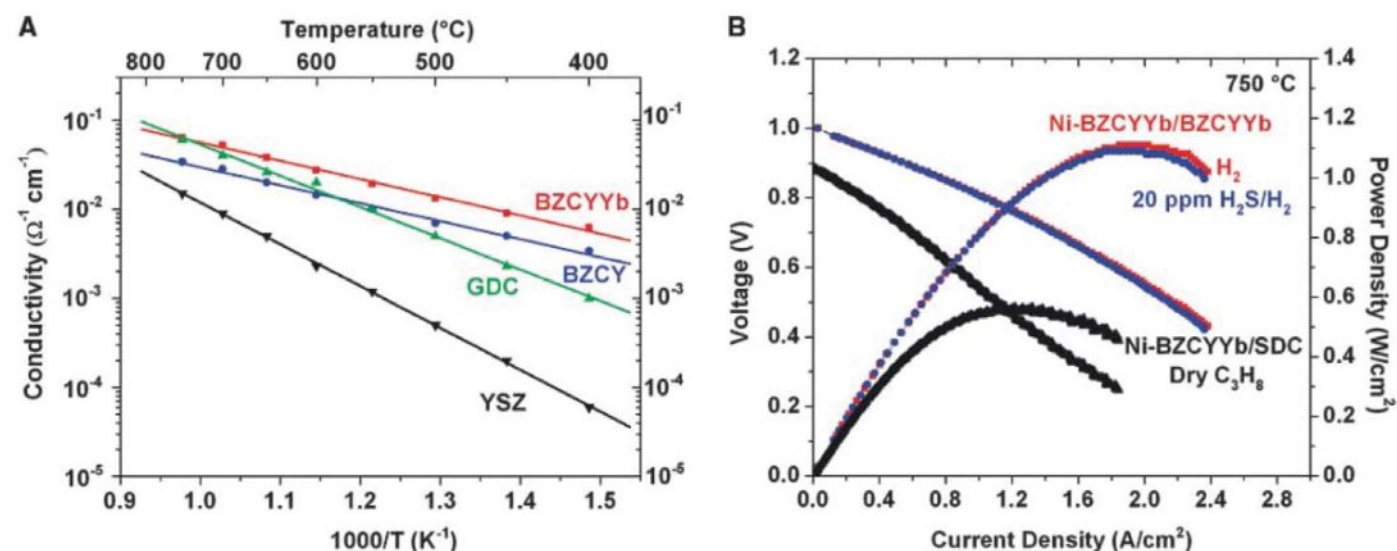


Fig. 1. (A) Ionic conductivities of BZCYYb, BZCY, GDC, and YSZ as measured at 400° to 750°C in wet oxygen (with ~3 vol % H_2O). (B) Typical current-voltage characteristics and the corresponding power densities measured at 750°C for a cell with a configuration of Ni-BZCYYb | BZCYYb |

BZCY-LSCF when ambient air was used as oxidant and hydrogen as fuel (with or without 20 ppm H_2S contamination), and for another cell with a configuration of Ni-BZCYYb | SDC | LSCF when dry propane was used as fuel.

Alternative anode materials and innovative anode structures have improved the tolerance to coking and poisoning by contaminants. To date, however, an initial drop of substantial magnitude in power output upon exposure to H_2S -contaminated fuels still appears unavoidable for any known anode materials. For example, the power output of a cell with $\text{Sr}_{0.8}\text{La}_{0.2}\text{TiO}_3$ as the anode-side support (which represents the latest advance in development of sulfur-tolerant anodes) dropped $\sim 19\%$ upon exposure to 100 ppm $\text{H}_2\text{S}/\text{H}_2$, although there was no additional degradation in performance under steady-state operation and the performance was fully recovered upon removal of H_2S (12).

We report high tolerance to coking and H_2S poisoning of a new material for SOFCs, a barium zirconate-cerate codoped with Y and Yb: $\text{BaZr}_{0.1}\text{Ce}_{0.7}\text{Y}_{0.1}\text{Yb}_{0.1}\text{O}_{3-\delta}$ (BZCYYb). This material has very high ionic conductivity below 750°C (Fig. 1A), which allows for fabri-

cation of anode-supported thin-electrolyte SOFCs of high power output at lower temperatures. There is no observable change in power output when the fuel is switched to one contaminated with 50 ppm H_2S , which we attributed to catalytic conversion of H_2S to SO_2 . Moreover, continuous and stable operation in dry propane for more than 100 hours without observable degradation in performance suggests that it is very effective for in situ reformation of hydrocarbons, which should help inhibit coking. Finally, it demonstrates adequate chemical and electrochemical stability over a wide range of conditions relevant to SOFC operation, implying long-term stability and long operational life. We also systematically investigated anodes consisting of Ni and BZCYYb in H_2S -contaminated H_2 and hydrocarbon fuels. They displayed not only impressive power output in clean hydrogen but also superior tolerance to coking and sulfur poisoning.

Doped zirconate-cerate compounds have been reported to exhibit both proton and oxide ion conductivity as well as a strong tendency for water absorption (or hydration) (13–17). For example, Y-doped zirconate-cerate, $\text{Ba}(\text{Zr}_{0.1}\text{Ce}_{0.7}\text{Y}_{0.2})\text{O}_{3-\delta}$ (BZCY), has high ionic conductivity and excellent chemical stability in atmospheres containing CO_2 and H_2O under SOFC operating conditions (18). Here, we explored codoping barium zirconate-cerate with Y and Yb using compositions of $\text{BaZr}_{0.1}\text{Ce}_{0.7}\text{Y}_{0.2-x}\text{Yb}_x\text{O}_{3-\delta}$ ($x = 0$ to 0.2). We believe that the two dopants on the B-site function in a cooperative fashion to improve the ionic conductivity and the catalytic activity for reforming or oxidation of hydrocarbons as well as conversion of H_2S to SO_2 .

When we compared the conductivities of BZCYYb with those of several other SOFC electrolyte materials, namely YSZ, GDC, and BZCY (Fig. 1A), BZCYYb displayed the highest conductivity below 750°C . Operation of SOFCs

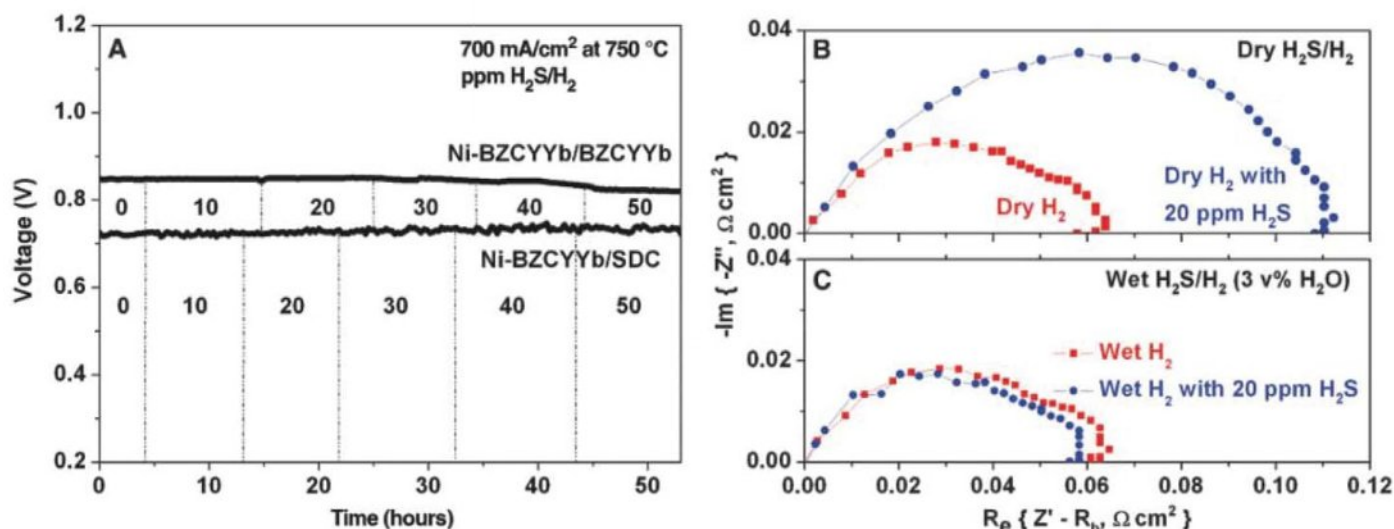


Fig. 2. (A) Terminal voltages measured at 750°C as a function of time for two cells with a configuration of Ni-BZCYYb | BZCYYb | BZCY-LSCF and Ni-BZCYYb | SDC | LSCF operated at a constant current density of $700 \text{ mA}/\text{cm}^2$ as the fuel was switched from clean H_2 to H_2 contaminated with different concentrations of H_2S (the number in each time interval represents

the concentration of H_2S in wet hydrogen in ppm). (B and C) Impedance spectra measured under OCV conditions at 750°C for a cell with a configuration of Ni-BZCYYb | SDC | LSCF in clean H_2 and in H_2 contaminated with 20 ppm H_2S for dry $\text{H}_2\text{S}/\text{H}_2$ gases (B) and wet $\text{H}_2\text{S}/\text{H}_2$ gases (with ~ 3 vol % H_2O) (C).

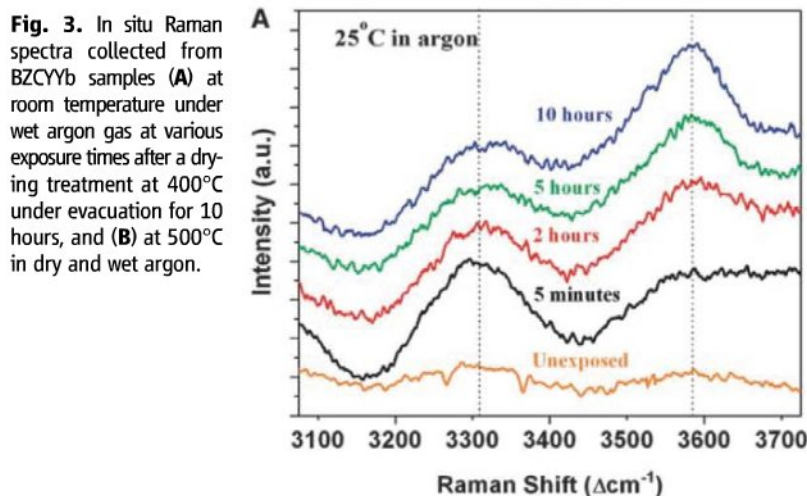


Fig. 3. In situ Raman spectra collected from BZCYYb samples (A) at room temperature under wet argon gas at various exposure times after a drying treatment at 400°C under evacuation for 10 hours, and (B) at 500°C in dry and wet argon.

at lower temperatures makes it possible to use much less expensive materials for cell components, thus reducing the cost while prolonging the operational life. As expected, the conductivities are sensitive to doping and partial pressure of oxygen, hydrogen, and water (figs. S1 and S2). When used as the electrolyte in a SOFC, the electronic conductivity of BZCYYb is relatively small, more so at lower temperatures (fig. S3). When used as a component for the anode and exposed to hydrogen and water, however, not only the ionic defects (OH_i° and $\text{V}_\text{O}^{\bullet\bullet}$) but also the electronic defects (e' and h') may coexist, enhancing the catalytic activity for reforming and/or oxidation of hydrocarbons and for conversion of H_2S to SO_2 . Although the conductivity of BZCYYb is about 80% better than that of BZCY (at 750°C), the catalytic activities of BZCYYb are quite different from that of BZCY. Under open circuit conditions, for example, the cells with a Ni-BZCYYb anode show higher and more stable open cell voltages (OCVs) than the cells with a Ni-BZCY anode when exposed to wet propane at 750°C (fig. S4), an indication of much-improved ability of hydrocarbon reformation.

Typical performance of a cell based on a Ni-BZCYYb cermet anode, a BZCYYb electrolyte, and a composite cathode consisting of BZCY and LSCF (19) (fig. S5) entailed peak power densities of $\sim 1.1\text{ W}/\text{cm}^2$ at 750°C (Fig. 1B) and $\sim 660\text{ mW}/\text{cm}^2$ at 650°C (fig. S6) when H_2 and ambient air were used as fuel and oxidant, respectively. Further, when the fuel was switched to H_2 contaminated with 20 ppm H_2S , the observed power output remained about the same. Moreover, a cell based on a Ni-BZCYYb anode and a $\text{Sm}_{0.1}\text{Ce}_{0.9}\text{O}_{2-\delta}$ (SDC) electrolyte displayed a peak power density of $564\text{ mW}/\text{cm}^2$ at 750°C when dry propane was used as the fuel, showing a considerably high resistance to coking and power output for a SOFC running on dry hydrocarbon. Typically, water has to be fed with a hydrocarbon fuel to prevent coking; however, addition of water will reduce the energy efficiency while increasing the complexity and cost of fuel cell systems (because it dilutes the fuel and requires a water management subsystem).

We investigated the sensitivity of this anode to sulfur poisoning by gradually increasing the concentration of H_2S in hydrogen. The terminal voltages of the same cells (with BZCYYb and SDC as the electrolyte) at 750°C were recorded as a function of time when the fuel was contaminated with different concentrations of H_2S (Fig. 2A). The Ni-BZCYYb anodes for both cells showed no observable change in power output as the fuel was switched from clean hydrogen to hydrogen contaminated with 10, 20, or 30 ppm H_2S . This sulfur tolerance was also evident from our impedance data (Fig. 2, B and C). When water was absent from the fuel, the electrode polarization resistance increased about 80% upon exposure to 20 ppm H_2S , from $\sim 0.06\text{ ohm}\cdot\text{cm}^2$ in H_2 to $\sim 0.11\text{ ohm}\cdot\text{cm}^2$ in H_2 containing 20 ppm H_2S , as commonly observed in previous studies (20). When a small amount of water (only $\sim 3\text{ vol } \%$) was introduced with the fuel, the electrode polarization resistance in hydrogen with 20 ppm H_2S was reduced to that in clean hydrogen, as can be interpreted from the collected impedance spectra (Fig. 2C). It is believed that sulfur poisoning is caused by the strong adsorption of the elemental sulfur on Ni surface and the three-phase boundaries (TPBs) between Ni, electrolyte, and the fuel. Sulfur would then block the active site for fuel oxidation in a traditional Ni-YSZ anode and increase the polarization resistance (21, 22). We hypothesized that water might adsorb on the surface of BZCYYb to facilitate the oxidation of H_2S or elemental sulfur to SO_2 at or near the active sites. Unlike H_2S or elemental sulfur, SO_2 readily desorbs from electrode surface (23).

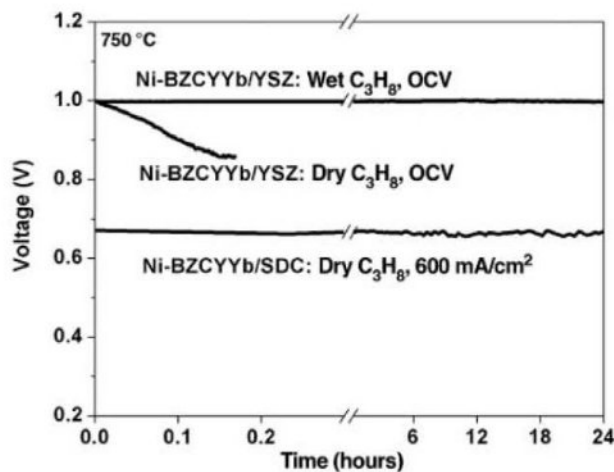
When the fuel was switched to hydrogen containing 40 and 50 ppm H_2S , the cell with a BZCYYb electrolyte (a mixed proton and oxide ion conductor) suffered some drop in power output, whereas the cell with an SDC electrolyte (an oxide ion conductor) displayed no observable change in performance. One possible explanation is that more water was produced at the active sites on the anode of the cell with an SDC electrolyte under active operation because of increased hydrogen oxidation; the water produced at the

active sites is beneficial to sulfur removal. Continuous operation in H_2S -contaminated fuel for a longer period of time (200 to 1000 hours) further confirmed that sulfur poisoning can be fully suppressed in the presence of a small amount of steam (figs. S7 and S8).

We used Raman spectroscopy to probe for the presence of water on the surface of BZCYYb. Previous studies suggest that the characteristic OH-bond vibration modes for water appear in the 3100 to 3700 cm^{-1} regime (24, 25). We collected Raman spectra from BZCYYb at different times of exposure to wet argon ($\sim 3\text{ vol } \%$ water) at room temperature, where incorporation of water into the bulk phase of BZCYYb is unlikely or negligible because of limited bulk diffusion. The BZCYYb powder sample was first dried at 400°C under evacuation for 10 hours to remove water from the sample. Upon exposure to wet ($3\text{ vol } \%$ H_2O) argon at room temperature, the mode near 3580 cm^{-1} emerged and slowly grew more intense over time. The presence of the modes in the 3100 to 3700 cm^{-1} range, particularly the mode that peaks near 3580 cm^{-1} (Fig. 3A), is strongly indicative of surface water molecules, as water modes at higher wavenumbers may correspond to adsorbed water with weak hydrogen bonds (26). Thus, one possible explanation for this behavior is that an initial layer of water accumulates at the surface quickly in a wet atmosphere, whereas any further water that adsorbs onto this layer builds up more slowly. These features are notably absent from the sample exposed to dry gas. Spectra collected in situ from samples held under the same gas conditions at 500°C display similar features and contrast (Fig. 3B). Because most fuels (including H_2) are humidified at room temperature (yielding $\sim 3\text{ vol } \%$ H_2O in gas) for typical operation of SOFCs, no excess water is needed to achieve the desired tolerance to H_2S contaminants.

The coking resistance of this material was demonstrated in a cell with a Ni-BZCYYb cermet anode, YSZ electrolyte, and LSCF cathode. When dry propane was used as fuel, the OCV dropped quickly within minutes (Fig. 4), an indication of rapid carbon deposition as expected for a conventional Ni-based anode. In contrast, when wet ($\sim 3\text{ vol } \%$ steam) propane was used as fuel, the OCV was very stable (Fig. 4), suggesting that the observed tolerance to coking is also attributed to the presence of a small amount of steam. Further, the contamination of Ni surface by BZCYYb during co-firing of BZCYYb and NiO might enhance the resistance to carbon buildup (fig. S9). The whole surface of the Ni-BZCYYb anode exposed to wet ($\sim 3\text{ vol } \%$ H_2O) propane appeared clean and free of carbon deposition, as revealed by Raman spectroscopy (fig. S10). The OCVs of the cell are greater than those observed for other alternative anode materials (5, 6, 9, 27) and approach 1.00 V for wet propane. Coking may be inhibited by water reforming propane on the surface of BZCYYb, and the reforming products (H_2 and

Fig. 4. Open cell voltages measured at 750°C as a function of time for a cell with a configuration of Ni-BZCYYb | YSZ | LSCF with dry and wet propane as the fuel, and the terminal voltages for another cell with a configuration of Ni-BZCYYb | SDC | LSCF operated at $600\text{ mA}/\text{cm}^2$ using dry propane as fuel. Stationary air was used as oxidant in all cases.



CO) adsorb on the active sites of the anode surface, leading to a stable OCV output. We note that conventional Ni-YSZ and Ni-GDC anodes suffered severe carbon deposition under the same conditions (fig. S11).

This catalytic activity of Ni-BZCYYb for in situ reforming of hydrocarbons was further demonstrated in operating cells powered by propane. A stable power output was observed for more than 100 hours (fig. S12) at a current density of 300 mA/cm² for a cell with a Ni-BZCYYb anode, a YSZ electrolyte, and an LSCF cathode when wet (~3 vol % steam) propane was used as fuel and air as oxidant. Analysis of the effluent gas by mass spectrometry indicated that propane was mostly converted to C₂H₄, CH₄, H₂, H₂O, CO₂, and CO through cracking, reformation, and electrochemical oxidation during cell operation (fig. S13). In contrast, conventional Ni-YSZ anodes studied under similar conditions showed rapid degradation in performance within minutes as a result of severe carbon deposition.

Because steam is produced on the anode surface of an operating cell when an oxygen anion conductor (e.g., SDC) is used as the electrolyte, tolerance to coking of BZCYYb-based anodes may be further enhanced by an operating current of such a fuel cell. Indeed, when the operating current density is sufficiently high, cells based on an SDC electrolyte (Fig. 4) can even operate in dry propane, producing stable power output without observable degradation. Microanalysis of the anode before and after operation in dry propane for 24 hours indicates that there was no visible change in microstructure and no observable carbon deposition (fig. S14). It appears that in situ reformation of hydrocarbons has prevented carbon deposition on the anode when an adequate

amount of water is produced at the active sites under the operating conditions.

To examine the chemical stability, we exposed BZCYYb powder samples to H₂ containing 50 vol % CO₂ or 50 vol % H₂O at 750°C for 300 hours and to H₂ containing 50 ppm H₂S at 750°C for 50 hours. X-ray diffraction (XRD) analysis of the samples before and after the exposures confirmed that BZCYYb is chemically stable under these testing conditions (figs. S15 and S16). Raman spectra were also collected from BZCYYb powder samples under wet and dry conditions in the 100 to 1000 cm⁻¹ range in order to assess the phase stability of the material with adsorbed water, and no compositional difference was apparent between the wet and the dry samples (fig. S17). Further, the Ni-BZCYYb anode is very stable under repeated electrochemical cycling: The terminal voltage of the cell was swept between OCV and 0.4 V at a rate of 10 mV/s for 1000 cycles with no appreciable change in peak power density (fig. S18). The remarkable ionic conductivity and stability suggest that BZCYYb is an attractive electrolyte or electrode component for low-temperature SOFCs. In addition, the material may also be used as a catalyst for reforming of hydrocarbon fuels and for removal of fuel gas contaminants such as sulfur.

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28. Supported by U.S. Department of Energy Basic Energy Science Catalysis Science Program grant DE-FG02-06ER15837.

Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/126/DC1
Materials and Methods
SOM Text
Figs. S1 to S18
References

10 April 2009; accepted 7 August 2009
10.1126/science.1174811

Rapid Resurgence of Marine Productivity After the Cretaceous-Paleogene Mass Extinction

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The course of the biotic recovery after the impact-related disruption of photosynthesis and mass extinction event at the Cretaceous-Paleogene boundary has been intensely debated. The resurgence of marine primary production in the aftermath remains poorly constrained because of the paucity of fossil records tracing primary producers that lack skeletons. Here we present a high-resolution record of geochemical variation in the remarkably thick Fiskeler (also known as the Fish Clay) boundary layer at Kulstirenden, Denmark. Converging evidence from the stable isotopes of carbon and nitrogen and abundances of algal steranes and bacterial hopanes indicates that algal primary productivity was strongly reduced for only a brief period of possibly less than a century after the impact, followed by a rapid resurgence of carbon fixation and ecological reorganization.

At the Cretaceous-Paleogene boundary (KPB) 65 million years ago, an asteroid impact was associated with mass extinction and widespread disruption of photosynthesis (1–3), possibly because of a reduction in incoming solar radiation caused by atmospheric debris

and sulfate aerosols (3, 4). Scenarios proposed for the immediate aftermath include a “Strangelove ocean,” where primary productivity was suppressed for a substantial interval of time (5), or an alternative “living ocean,” where there was little interruption of productivity and, instead, the

flux of organic matter from the surface to the deep ocean ceased in the postextinction ocean (2, 6). Model simulations suggest that the physical effects of reduced sunlight may have lasted no longer than a decade (4). It is assumed that pelagic ecosystems required up to ~3 million years to fully recuperate (2, 6), although continental margin ecosystems seemed to have recovered rather quickly (7, 8), possibly because opportunistic neritic species with benthic cysts or resting stages may

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have selectively survived (9–11), particularly at high latitudes (12). However, processes taking place in the immediate aftermath remain poorly understood. Fossils are scarce and interpretations further compromised by poor preservation and reworking in the cosmopolitan boundary clay layer (13, 14), which presumably was deposited in $\sim 10,000 \pm 2000$ years (15). This interval is hypothesized to represent the duration required for the restoration of food chains and ecosystems after the impact (15). However, many planktonic organisms are devoid of preservable hard parts, including microorganisms at the basis of the marine food chain, such as most microalgae and cyanobacteria.

Here we present high-resolution geochemical and biomarker-based records (16) of a 37-cm-thick section of the Fiskeler clay layer [also known as the Fish Clay (FC)] at Kulstirenden, Stevns Klint, Denmark, an expanded counterpart of the renowned section at Højerup (13, 17) (fig. S1). Hydrocarbon biomarkers obtained from the Fiskeler show evidence of low-to-moderate biodegradation (fig. S3). Using a sensitive mass spectrometry technique, we focused on biomarkers that are resistant to biodegradation, such as C_{27-29} steranes and C_{27-35} hopanes [supporting online material (SOM)]. Well-preserved and thermally immature bitumens found within the bottom 16 cm of the FC (gray area in Fig. 1; figs. S4 and S5) contrast strongly with previously fossilized and thermally mature organic matter before the boundary, in the upper 20 cm of the Fiskeler, and in the Cerithium Limestone that had entered the system through weathering of local sedimentary rocks. This stark maturity contrast in a continuously deposited sedimentary sequence is *prima facie* evidence for mixtures of organic matter of differing ages and thermal histories (18). Bitumen isolated from the lower 16 cm of the Fiskeler contains a higher proportion of autochthonous, thermally immature organic matter comprising the preserved remains of organisms present in the postextinction water column, probably as a result of increased organic matter preservation under oxygen-depleted conditions and an enhanced clay content in the sediments. The preservation of autochthonous organic matter in late Maastrichtian chalk was probably poor in this richly oxygenated and well-mixed, shallow-water depositional environment (13, 14). The proportion of allochthonous biomarkers increased abruptly ~ 17 cm above the boundary and reaches pre-boundary values at ~ 27 cm (Fig. 1). This trend is interpreted as the return to a depositional environment where organic matter is vigorously remineralized, and it occurs before the full recuperation of carbonate sedimentation (fig. S2). Therefore, the bottom 16 cm of the Fiskeler provides an exceptional opportunity for studying the ecological recovery after the KPB using biomarkers.

The carbon isotopic composition of bulk carbonate ($\delta^{13}C_{carb}$) from below the boundary is lower than that seen in earlier observations on fine-fraction carbonate (13). At the boundary, $\delta^{13}C_{carb}$ shifts by about +0.5 per mil (‰) and

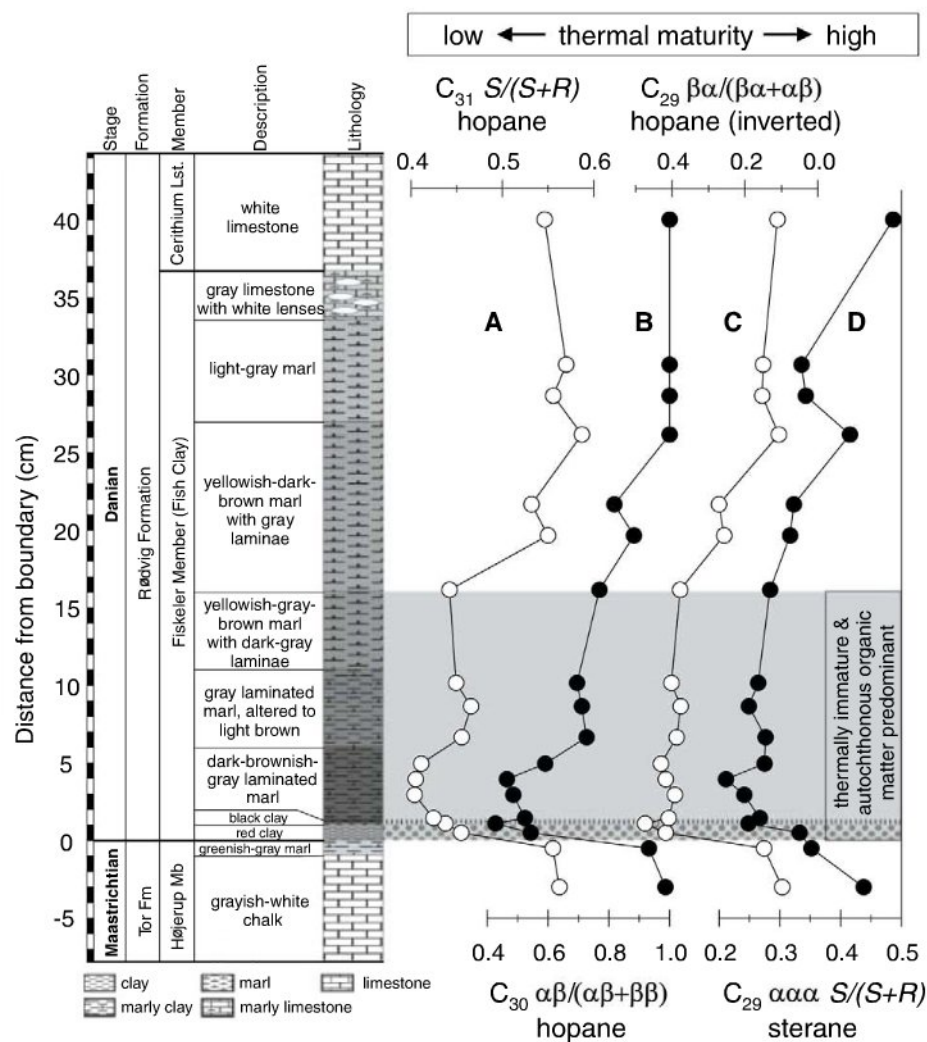


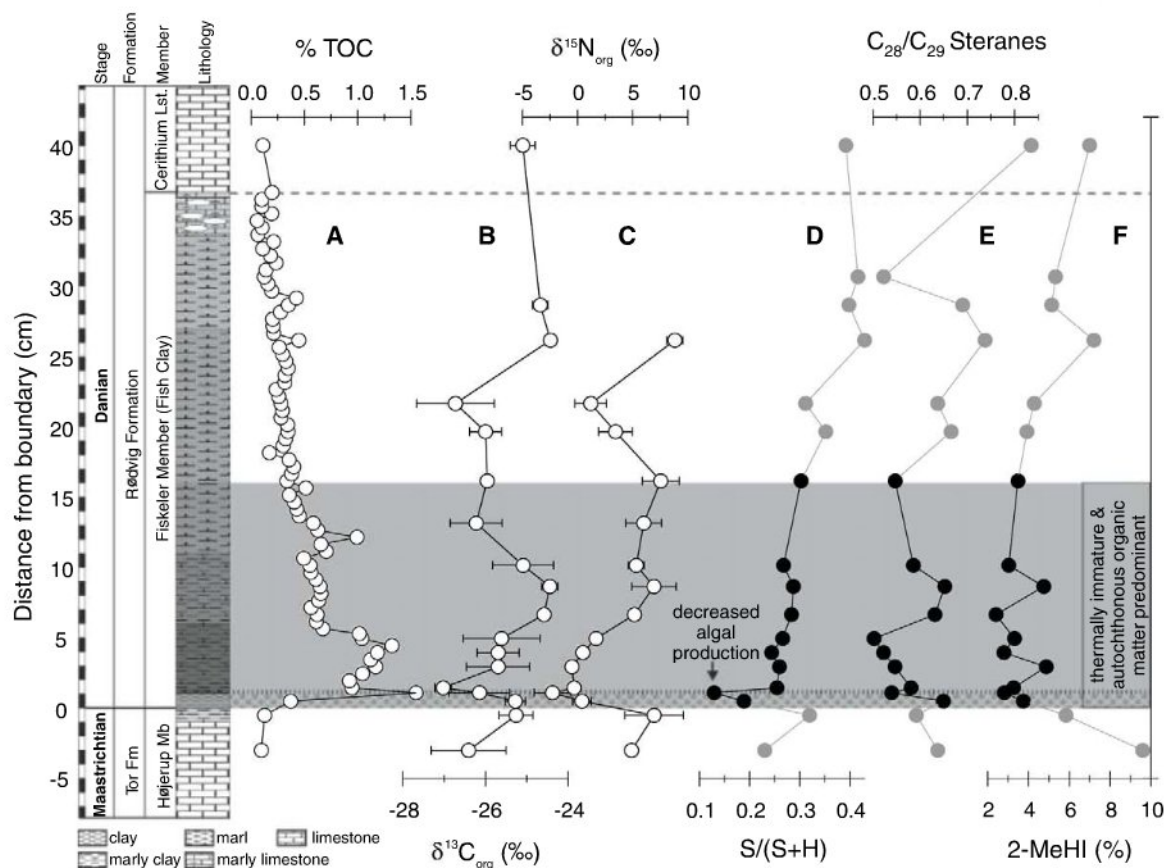
Fig. 1. Stratigraphy and biomarker-based thermal maturity indexes of the Kulstirenden section. (A) C_{31} S/(S+R) hopane ratio; (B) C_{30} $\alpha\beta/(\alpha\beta+\beta\beta)$ hopane ratio; (C) C_{29} $\beta\alpha/(\beta\alpha+\alpha\beta)$ hopane ratio (inverted scale); (D) C_{29} $\alpha\alpha\alpha$ S/(S+R) sterane ratio. The gray area denotes the predominance of stereoisomers with the original biologically inherited stereochemistry, and thus the presence of thermally immature autochthonous organic matter within the lower 16 cm of the Fiskeler suitable for interpretation of biomarkers sources. Shaded areas represent the red clay (dark gray circles) and the black clay (vertical lines).

then remains rather uniform, with a slight and steady shift toward lower values (fig. S2). The stable isotopic compositions of C and N in kerogen, $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$, respectively, are lowest in the black clay and the lowest part of the Fiskeler (Fig. 2). We suggest that these coupled negative excursions reflect the post-impact shutdown and early stages of recovery of primary productivity. Accordingly, diminished availability and biological assimilation of nitrate resulted in minimal N-isotopic fractionation (19), and the associated minimal algal growth rate led to maximum C-isotopic fractionation (20). As at other boundary sections (7, 21), this early period of the deposition of the clay layer was probably associated with oxygen-depleted conditions, at least in bottom waters. This is supported by the presence of noticeable laminations (fig. S1), a relatively high total organic carbon (TOC) content (Fig. 2), and pyrite with stable S isotope

values indicative of intense microbially mediated sulfate reduction (22). Thus, nitrate depletion due to denitrification under oxygen-depleted waters is likely to have occurred. The steady increase of $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ above the black clay is consistent with a progressive recovery of the ecosystem's productivity on a centennial-to-millennial time scale if we assume that the sedimentation rate was constant over the entire $\sim 10,000$ years of clay deposition (table S2 and SOM).

The presence of C_{27-29} algal steranes, C_{30} 4-methyl steranes and dinosteranes, and bacterial triterpenoids indicates the presence of "normal" marine organic matter (fig. S4) and suggests that photosynthesis, although diminished, persisted. The contribution of terrestrial organic matter appears to be minor (SOM). The low concentration of steranes in the black clay (fig. S6) and a notably low steranes/(steranes+hopanes) ratio [S/(S+H)] (Fig. 2) is probably the result of reduced

Fig. 2. Bulk geochemistry and biomarker-based source indexes. (A) TOC content. (B) C isotopic composition of kerogen. (C) N isotopic composition of kerogen. (D) C_{27-29} steranes/ $(C_{27-29}$ steranes + C_{30-35} hopanes) ratio, indicative of relative changes among eukaryotic and bacterial sources. (E) C_{28} relative to C_{29} steranes, indicating the contribution of chlorophyll c algae relative to chlorophyll a and -b algae (28); (F) 2-MeHI, indicative of the relative contribution of cyanobacteria (29). Black circles in (D), (E), and (F) designate samples with predominantly autochthonous biomarkers suitable for interpretation in an ecological context. Gray circles, in contrast, represent samples with strong allochthonous contributions to the biomarker record (compare with Fig. 1); therefore, the resulting values are not directly comparable to samples indicated by black circles. Error bars represent SD, and gray and shaded areas correspond to those in Fig. 1.



algal production while reduced sunlight transmission and nitrate-limiting conditions prevailed (SOM). Although a low $[S/(S+H)]$ could also be related to increased prokaryotic input, such as observed in the Permian-Triassic boundary (PTB) (23), the concentration profiles of both hopane and steranes strongly suggest that this is not the case (fig. S6). An immediate increase of $[S/(S+H)]$ in the following millimeters indicates a rapid resurgence of algal production, as suggested to have occurred once optimal incoming solar radiation levels returned (1, 2). However, $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ data suggest that initially, primary production remained subdued (Fig. 2). Algal communities may have included photosynthetic microbiota capable of producing resting structures (9, 10) and/or opportunistic organisms capable of adapting quickly to environmental changes by, for example, switching between autotrophic and heterotrophic metabolism [mixotrophy (24)]. Although an early recovery of primary production has been suggested on the basis of microfossil (11, 25), isotopic (26), and geochemical records (7, 8, 25), estimates of its timing vary enormously. In view of the rapid sedimentation of the entire clay layer [$\sim 10,000$ years (table S2) (8, 15, 27)], and considering several scenarios of sedimentation, the deposition of the 2-mm-thick black clay layer at Kulstirrenden could have occurred on a decadal-to-centennial time scale (SOM), which is the period corresponding to maximal disruption of

primary production after the deposition event of the fallout lamina.

Low values of the $C_{28}/(C_{28}+C_{29})$ sterane ratio of less than 0.6 at the base of the Fiskeler (Fig. 2) reveal a diminished contribution of chlorophyll c-containing eukaryotic phytoplankton, such as diatoms, dinoflagellates, and coccolithophorids (C_{28}) relative to green algae (C_{29}) (28). These low values reflect the extinction of groups of phytoplankton and an overall diminished productivity at the boundary because they strongly differ from those in well-studied petroleum samples from the Cenozoic (>0.9) and the second half of the Mesozoic (>0.8), when the geological record shows that the major expansion of chlorophyll c-containing algae took place (28). The presence of cyanobacterial 2-methyl hopanes throughout the section (fig. S5), combined with the low variability of the 2-methyl hopane index (2-MeHI) (29) (Fig. 2), suggest that cyanobacteria were not affected in the same manner as eukaryotic algae and, by inference, indicates that variations in cyanobacterial productivity did not substantially influence the main shift observed in $[S/(S+H)]$. Values of the 2-MeHI between 2 and 5 at the base of the FC are typical for mid- to high-latitude Phanerozoic sedimentary rocks (28, 30), implying that productivity levels of 2-methylhopane-producing cyanobacteria were normal. However, these values are low compared to those reported for the PTB (23) and

oceanic anoxic events (30), when N_2 -fixing cyanobacteria flourished, implying that it was not enhanced N_2 fixation but decreased eukaryotic productivity that was responsible for low $\delta^{15}N$ values at the boundary (Fig. 2).

Our study shows that primary algal production in a neritic high-latitude ecosystem rapidly recovered after the impact event at the KP, evidencing a rapid biological turnover consistent with models suggesting a rapid resurgence of photosynthesis after solar radiation levels were restored (2, 4). Yet the global implications of our findings from a single locality remain to be verified at other boundary-clay layers worldwide.

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31. This research was funded by the Deutsche Forschungsgemeinschaft through the European Graduate

College EUROPROX and MARUM, and NASA (Astrobiology Institute, Exobiology Program). We thank X. Prieto, C. Colanero, L. Sherman, J. Salacup, H. Buschhoff, M. Segl, U. Beckert, P. Simundic, C. Gonzalez, A. Kelly, B. Schmincke, M. Milling-Goldbach, and A. Skorokhod for their laboratory and/or logistic assistance. R.E.S. acknowledges the Hanse-Wissenschaftskolleg and the Alexander von Humboldt-Stiftung. J.S. acknowledges EUROPROX for supporting his doctoral studies and a research visit to MIT.

Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/129/DC1

Materials and Methods

SOM Text

Figs. S1 to S6

Tables S1 and S2

References

12 May 2009; accepted 10 August 2009

10.1126/science.1176233

Identification of Carboniferous (320 Million Years Old) Class Ic Amber

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The presence of amber, the fossil form of the resins produced by many types of higher plants, has been reported from many localities in Mesozoic and Cenozoic rocks. We have found Class I (polylabdanoid) amber in Carboniferous sediments dating to ~320 million years ago. This result demonstrates that preconifer gymnosperms evolved the biosynthetic mechanisms to produce complex polyterpenoid resins earlier than previously believed and that the biosynthetic pathways leading to the types of polylabdanoid resins that are now typically found in conifers and those now typically found in angiosperms had already diverged by the Carboniferous.

Terpenoid resins are produced by nearly all modern conifers and by many angiosperms. These products serve a variety of ecological functions, including sealing and protecting wounds, repelling insects, and discouraging herbivores. (1). Many resins contain terpenoid

compounds that readily polymerize when exposed to light or air (1), resulting in the formation of the hardened resinous masses commonly observed on modern trees. Solidified resins are often highly resistant to many typical degradation mechanisms and are commonly preserved in sediments. The fossil form of plant resin is known as amber. Ambers are classified on the basis of the chemical nature of the polymerized terpenoids composing the macromolecular structure (2, 3). The most common class of ambers (Class I), based on po-

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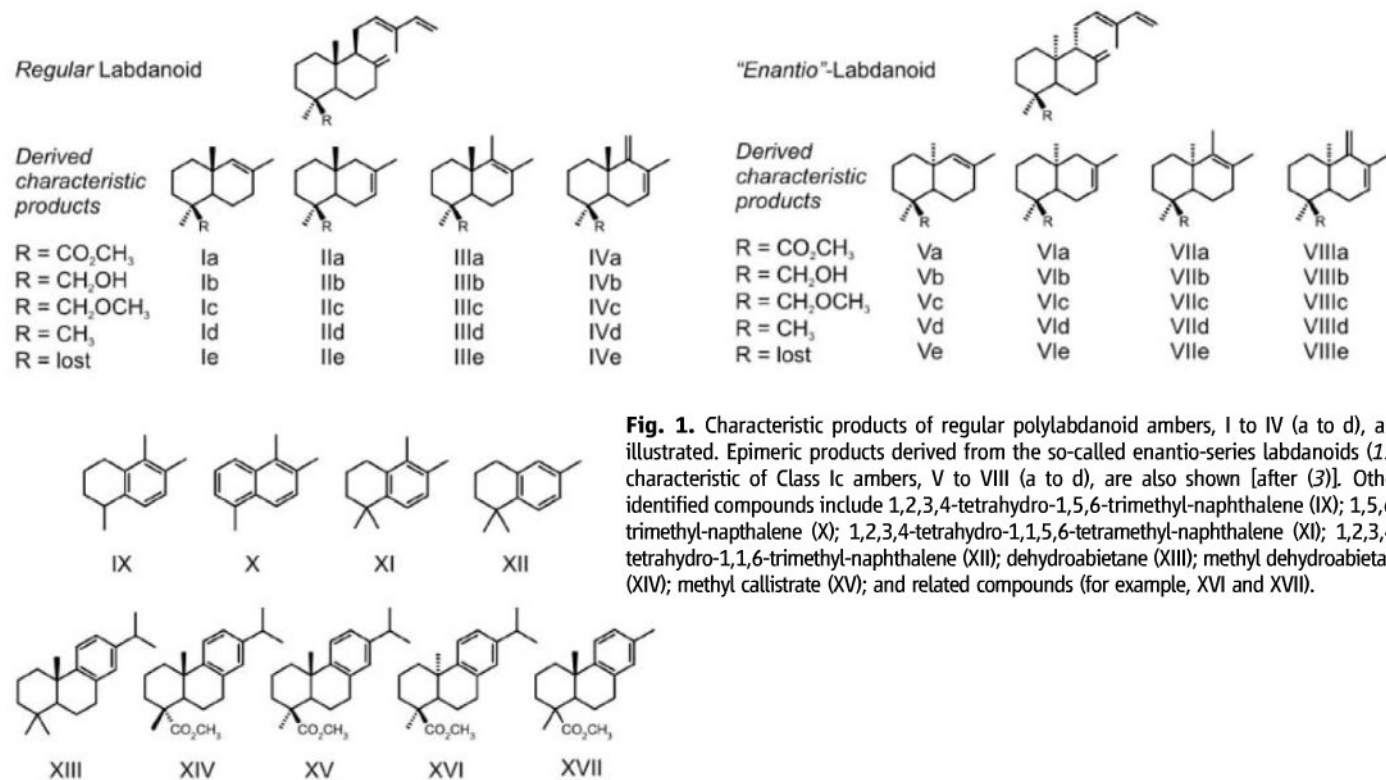


Fig. 1. Characteristic products of regular polylabdanoid ambers, I to IV (a to d), are illustrated. Epimeric products derived from the so-called enantio-series labdanoids (11) characteristic of Class Ic ambers, V to VIII (a to d), are also shown [after (3)]. Other identified compounds include 1,2,3,4-tetrahydro-1,5,6-trimethyl-naphthalene (IX); 1,5,6-trimethyl-naphthalene (X); 1,2,3,4-tetrahydro-1,1,5,6-tetramethyl-naphthalene (XI); 1,2,3,4-tetrahydro-1,1,6-trimethyl-naphthalene (XII); dehydroabietane (XIII); methyl dehydroabietate (XIV); methyl callistate (XV); and related compounds (for example, XVI and XVII).

lymerized labdanoid diterpenes, has been shown to exist since at least the early Cretaceous (4). Previous investigations of unequivocal pre-Cretaceous terpenoid ambers have been limited to spectroscopic techniques, such as infrared spectroscopy or nuclear magnetic resonance (5–7). These analyses only survey the functional groups present and do not yield definite chemical structures. Previous pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) analyses on equivocal Carboniferous resin rodlets and resinite separated from coal resulted in waxy compositions (8–10) totally unlike the Class I terpenoid ambers presented here. Therefore, the chemical nature of ambers before the Cretaceous and the evolutionary development of terpenoid resin are unknown. Here we present Py-GC-MS analyses of macroscopic amber from Carboniferous [~320 million years old] coal, and demonstrate that this material is a mature Class Ic amber. This result demonstrates that pre-conifer gymnosperms evolved the biosynthetic mechanisms to produce complex polyterpenoid resins earlier than previously believed.

Class I ambers are subclassified on the basis of the stereochemical nature of the labdanoids that make up their macromolecular structure and on the presence or absence of succinic acid in the macromolecular structure (3). Two types of labdanoid diterpenes are found in Class I ambers: the so-called regular and enantio series (Fig. 1) (11). Regular polylabdanoid ambers containing succinic acid are classified as Class Ia (the abundant and well-known ambers from the Baltic region are generally of this class). Class Ib ambers are also based on regular polylabdanoids, but these do not incorporate succinic acid. This type of amber is the most common on a global basis. Class Ic ambers are based on polymers of enantio-series labdanoids and also lack succinic acid (3). In modern species, resins based on regular polylabdanoids, equivalent to Class Ib ambers, are commonly derived from conifers, whereas resins based on enantio-series labdanoids, equivalent to Class Ic ambers, are commonly associated with angiosperms (there is no known extant species that produces resin analogous to Class Ia ambers).

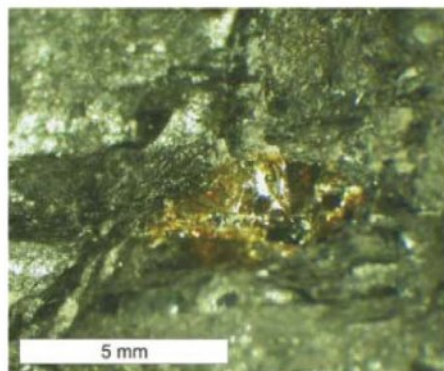


Fig. 2. Bleb of Carboniferous amber. The amber is brittle with resinous luster. Average bleb size is approximately 5 mm.

Nonpolylabdanoid ambers are also known, but are much less common than Class I ambers.

Pyrolysis of Class I ambers produces characteristic bicyclic products derived from the A/B ring system of the original labdanoid monomers (3) (Fig. 1, I to IV, a to d; or V to VIII, a to d). These characteristic products, which are readily identified by GC-MS, retain the original stereochemistry of the precursors and hence are a reliable means of identifying and differentiating Class Ia/Ib and Class Ic ambers.

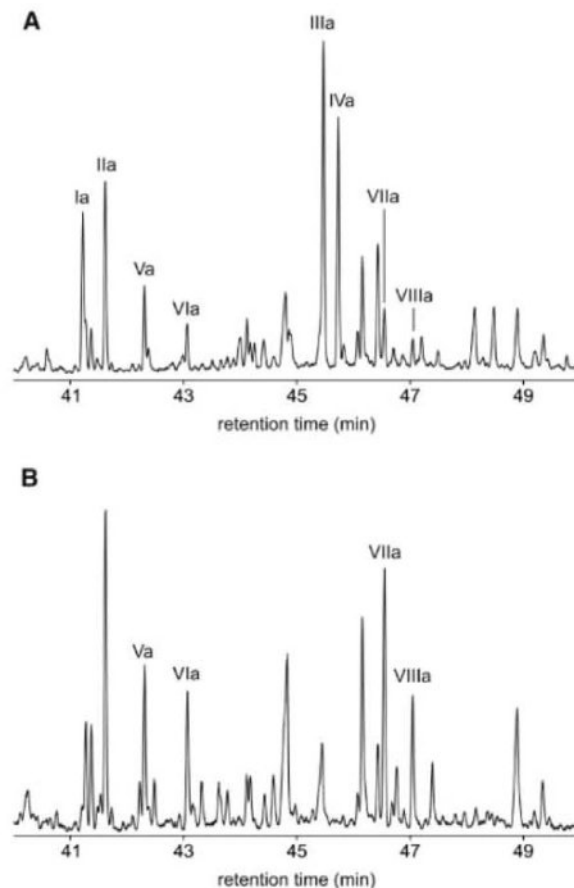
Polylabdanoid macromolecular resin structures are common across a wide range of taxa. However, it is also common for nonpolymerizable terpenes to occur as occluded products within the polylabdanoid structure (1, 3). The nature and distributions of these compounds, which may include abietanes, pimaranes, totaranes, and kuaranes, among many others, tend to vary more widely between families and genera. These diterpenes are frequently specific to the originating family, and therefore have substantial chemotaxonomic value in reconstructing paleoenvironments, as well as in assisting in the determination of phylogenetic relationships (12).

We have recovered well-preserved macroscopic blebs of amber (Fig. 2) from an Illinois coal seam located within the Lower Desmoinesian Series, Tradewater formation, which is stratigraphically dated to ~320 million years ago, and have analyzed these by Py-GC-MS. Amber blebs were

golden yellow and specimens were infrequent. The pyrolysates of these samples showed that they contain characteristic bicyclic products associated with the pyrolysis of Class I (labdanoid based) ambers (Fig. 1, V to VIII, a, d, and e). Methyl ether and alcohol products (Fig. 1, V to VIII, b and c) appear to be absent. These samples lack succinic acid and are composed of enantio-type labdanoids and are, therefore, classified as Class Ic ambers. Analyses of five discrete blebs resulted in nearly identical compound distributions, indicating that these ambers are all derived from the same botanical source. No amber specimens were found in association with plant macrofossils, and the botanical origin of these materials is as yet unknown.

The stereochemical nature of the labdanoids present in the macromolecular structure of these samples was determined by co-pyrolysis with the following well-characterized reference ambers: Raritan amber, Class Ib, regular labdanoids, coniferous origin (Cupressaceae), from New Jersey, United States (13); and Chiapas amber, Class Ic, enantio labdanoids, angiospermous origin (*Hymenaea*), from Chiapas, Mexico (3) (Fig. 3). Both regular and enantio labdanoid products are present in the co-pyrolysate of the Carboniferous amber and the Raritan amber (Fig. 3A), whereas only enantio products are observed in the co-pyrolysate of the Carboniferous amber with the Chiapas sample (Fig. 3B).

Fig. 3. Multiple ion traces of co-pyrolysis analyses. (A) Co-pyrolysis of Raritan amber (regular, Class Ib) with the Carboniferous amber and (B) co-pyrolysis of Chiapas amber (enantio, Class Ic) with the Carboniferous amber. Multiple ion traces were constructed using ions of mass-to-charge ratios of 161, 173, 175, and 176, corresponding to the methyl ester pyrolysis products of polylabdanoid ambers (Ia to VIIIa). All four characteristic products are present.



Several bicyclic compounds with structures similar to the characteristic products were also present in the pyrolysates, including 1,2,3,4-tetrahydro-1,5,6-trimethyl-naphthalene (Fig. 1, IX), trimethyl-naphthalenes (Fig. 1, X), 1,2,3,4-tetrahydro-1,1,5,6-tetramethyl-naphthalene (Fig. 1, XI), and 1,2,3,4-tetrahydro-1,1,6-trimethyl-naphthalene (Fig. 1, XII), among others. These compounds are interpreted to be the pyrolysis products of degraded labdanoids within the macromolecular structure and generally differ by having fewer methyl groups and/or by having increased aromaticity. The presence of these compounds is not surprising, considering the age and maturity of these samples.

In addition to the characteristic labdanoid products, several abietane-class diterpenes were also identified in the pyrolysates. These included dehydroabietane (Fig. 1, XIII), methyl dehydroabietate (Fig. 1, XIV), methyl callitrate (Fig. 1, XV), related compounds (Fig. 1, XVI and XVII α and β), and others. These compounds are common in Class I ambers (1, 3), as well as in modern resins (12). The distributions of these products in pyrolysates generated by pyrolysis at temperatures $T_{py} = 300^\circ\text{C}$ and $T_{py} = 480^\circ\text{C}$ are essentially identical, indicating that these diterpenes are present in these samples as occluded materials. The exact stereochemistry of dehydroabietane (Fig. 1, XIII) or methyl 16,17-dinor dehydroabietate and methyl 16,17-dinor callitrate (Fig. 1, XVII α and β) is unknown in these samples, but is drawn in Fig. 1 as commonly found in Class I ambers.

The observation of Class Ic ambers in Carboniferous sediments suggests that pre-conifer gymnosperms were using complex polyterpenoid resin in a manner similar to that seen in a wide variety of modern species. Modern resins that are structurally analogous to Class Ic ambers are primarily derived from angiosperms. Our data do not imply that angiosperms existed in the Carboniferous, because the fossil record does not record unequivocal angiosperm fossils until the Cretaceous. However, our data do suggest that the divergence of the biosynthetic mechanisms needed to produce resins based on regular and enantio-series labdanoid diterpenes predates both the emergence of true conifers and the differentiation of angiosperms and gymnosperms. Furthermore, these basic biosynthetic pathways have been retained in both gymnosperms and angiosperms through several major extinction events and over 300 million years of evolution. Based on genomic evidence, previous workers have postulated initial differentiation of terpene synthase genes associated with the production of resin-related diterpenes during the Carboniferous (14). Our data support this hypothesis.

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11. Despite the common terminology used to describe labdanoids related to communic acids as regular and those related to ozic acid as enantio, these products are in fact epimeric, not enantiomers. The same is true of the bicyclic products (Fig. 1, I to IV and V to VIII) derived from the polymers of these terpenoids by Py-GC-MS. This terminology, although imprecise, is deeply entrenched in the literature related to these compounds and is retained here for consistency with numerous previously published works.
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15. This contribution constitutes part 14 of the authors' series of publications under the general title, "The Nature and Fate of Natural Resins in the Geosphere."

Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/132/DC1

Materials and Methods

References

10 June 2009; accepted 11 August 2009

10.1126/science.1177539

Immune Activation by Life-Shortening *Wolbachia* and Reduced Filarial Competence in Mosquitoes

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Wolbachia strain *wMelPop* reduces the longevity of its *Drosophila melanogaster* host and, when introduced into the mosquito *Aedes aegypti*, halves its life span. We show that *wMelPop* induces up-regulation of the mosquito's innate immune system and that its presence inhibits the development of filarial nematodes in the mosquito. These data suggest that *wMelPop* could be used in the global effort to eliminate lymphatic filariasis and possibly for the control of other mosquito-borne parasites where immune preactivation inhibits their development. The cost of constitutive immune up-regulation may contribute to the life-shortening phenotype.

Wolbachia pipientis is a maternally inherited intracellular bacterium of invertebrates, capable of spreading itself through populations by reproductive manipulation such as cytoplasmic incompatibility (CI). The strain *wMelPop* or "popcom," unusually, reduces the longevity of its *Drosophila melanogaster* host (1) and also has been shown to halve life-span when the mosquito *Aedes aegypti* was stably trans-

infected (2). The *wMelPop* life-shortening phenotype offers the prospect of a disease control system by potentially skewing the population structure toward younger individuals. Vectorial capacity is particularly sensitive to mosquito age because mosquito-borne pathogens require an extrinsic incubation period between ingestion and transmission that is long relative to mean life-span in the field, such that only older mosquitoes within

a population are potentially infective. *wMelPop* was also found to be inherited at high rates and to induce strong CI in *Ae. aegypti*, which provides a reproductive advantage to infected females. The *wMelPop* strain should be capable of spreading through populations despite the reduction in mean life-span, because reproduction by older individuals makes a relatively small contribution to the next generation (2–6).

We compared host gene expression using whole-genome microarrays in genetically identical *Ae. aegypti* lines infected and uninfected with *wMelPop* (7) to examine the mechanism underlying the life-shortening phenotype. Of 199 gene transcripts up-regulated by more than a twofold threshold, 78 had putative immune-related functions (Fig. 1 and table S2). These included genes that encode 17 CLIP domain, serine proteases, nine FREPs (fibrinogen-related proteins), six cecropins, four TEPs (thioester-containing proteins), three defensins, three PPOs (prophenoloxidases), two lysozymes, two PGRPs (peptidoglycan recog-

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nitition proteins), two GNBP (Gram-negative binding proteins), and the nuclear transcription factor *Rel2*. Various effector genes, particularly cecropins and other antimicrobial peptides, show the highest up-regulation (Fig. 1 and table S2). Five immune-related genes (primarily of predicted regulatory function) were down-regulated below the twofold threshold (table S2).

Quantitative RT-PCR (qRT-PCR) experiments with mosquitoes at 2 and 15 days post eclosion using a subset of immune-related genes, provided broad support for the array data (Fig. 2 and table S1). Up-regulation occurred at similar levels at both age points. This scale of constitutive or chronic immune up-regulation is unexpected (the short-lived acute immune gene expression is commonly observed after septic challenge). In *Drosophila simulans* and *Aedes albopictus*, naturally occurring *Wolbachia* was found neither constitutively to induce nor to suppress the transcription of various inducible antibacterial genes (8). Constitutive up-regulation of immune genes is known to have a high cost, and trade-offs between longevity and pathogen resistance associated with immune up-regulation have been described in *D. melanogaster* (9). Therefore, constitutive up-regulation of immune genes associated with *wMelPop* infection also provides a

possible mechanism for, or contributory factor to, the life-shortening phenotype.

The development of various pathogens is known to be inhibited by immune preactivation in mosquitoes, which suggests that the constitutive immune regulation observed could influence the transmission of these pathogens to humans. For example, when *Ae. aegypti* was injected with bacteria 24 hours before challenge with *Brugia* filarial nematodes, causative agents of human lymphatic filariasis, prevalence and mean intensity of infection were reduced significantly (10). Synthetic cecropin peptides also have known filarial-killing activity (11); data from the microarray experiment showed that six cecropin genes were all up-regulated by more than 25-fold in the presence of *wMelPop* (table S2).

We investigated the effect of *wMelPop* on filarial transmission. A filarial susceptibility locus from the susceptible Ref^m strain of *Ae. aegypti* was backcrossed into the *wMelPop*-infected and control uninfected backgrounds by using a visible marker linked to the susceptibility locus (7). A blood meal infected with *Brugia pahangi* microfilariae (a rodent filarial nematode previously shown to be a good model for studies of the genetics of susceptibility to infection by human filariae) was provided (12). In comparison with the uninfected control line, significant reductions in the mean numbers of third larval stage (L3) infective worms, as well as in the prevalence of infected mosquitoes, were observed at three microfilarial densities when the *wMelPop* infection was present (Fig. 3). The inhibition effect was strongest at the lowest microfilarial density used, 11 microfilariae per microliter of blood, with 79.8% (A bars) or 84.2% (B bars) reductions in mean L3 stage larvae in the *wMelPop*-infected line compared with the *wMelPop*-uninfected control. All microfilarial densities used are high compared with blood densities that would occur naturally.

Filarial nematodes are somewhat harmful to mosquitoes. To investigate whether the presence of *wMelPop* conferred protective effects against other insect pathogens, the two lines were challenged by thoracic pricking with a virulent strain of the Gram-negative bacterium *Erwinia carotovora*. There was higher survival when *wMelPop* was present than in the *wMelPop*-free control (Fig. 4). A control challenge with the Gram-positive bacterium *Micrococcus luteus* confirmed that the high mortality seen when the *wMelPop*-uninfected line was challenged with *Erwinia* was not simply a result of septic injury.

Any protective effects provided against harmful pathogens commonly encountered by mosquitoes will act to increase the population-spreading capacity of the *wMelPop* strain. However, any benefits accrued from pathogen protection in nature are likely only partially to offset the fitness costs associated with immune gene expression. The immune activation phenotype may be a side effect of the unusually fast replication of *wMelPop* and/or because the immune evasion strategies normally used by *Wolbachia* are impaired in this novel host. *wMelPop Wolbachia* must itself be at least partly resistant to the immune effectors induced, because it is maternally transmitted at high levels in infected *Ae. aegypti* (2).

Wolbachia infections, including *wMelPop*, were recently reported to provide a protective function against pathogenic viruses in *Drosophila* (13, 14). The up-regulation of immune genes provides a possible explanation or contributory factor, because there is a degree of overlap between immune peptides induced by bacteria and viruses (15, 16). Cecropins have previously been shown to have antiviral effects, for example, inhibiting HIV-1 (human immunodeficiency virus 1) replication (17), and an artificial cecropin-defensin hybrid peptide inhibited dengue virus replication (18). Knockdown studies will allow

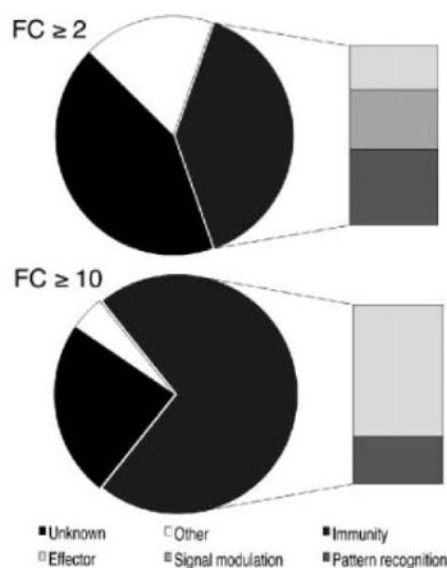
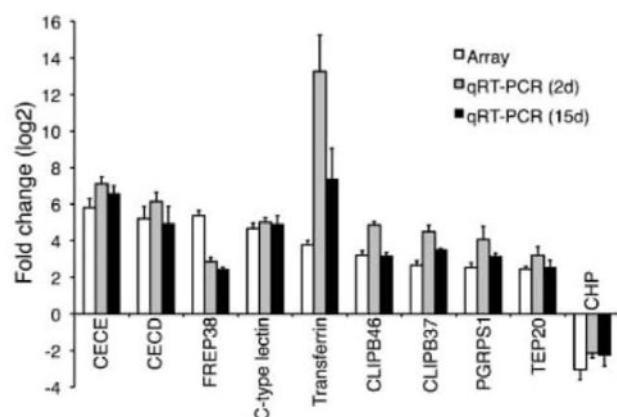


Fig. 1. Induction of immune gene transcription in *wMelPop*-infected female *Ae. aegypti*. A summary of data from four replicate *Ae. aegypti* microarrays is shown as gene transcripts significantly up-regulated in *wMelPop*-infected versus uninfected female mosquitoes. Of 199 up-regulated gene transcripts with fold change (FC) ≥ 2 ($P \leq 0.05$), a considerable proportion had a putative immune function (immunity) compared with genes of other known function (other) and unknown function (unknown). Gene transcripts with immune-related function were categorized into effectors, signal modulation, and pattern recognition. Gene transcripts encoding immune effectors dominated the list of highly up-regulated genes (FC ≥ 10).

Fig. 2. Microarray and qRT-PCR analyses of differentially regulated genes. The differential regulation of transcript levels in *wMelPop*-infected *Ae. aegypti* versus a genetically identical uninfected *Ae. aegypti* line was examined for a selection of nine up-regulated genes corresponding to two cecropins (CECE, AAEL000611; CECD, AAEL000598), a fibrinogen- or fibronectin-related protein (FREP38, AAEL0015428), a C-type galactose-specific lectin (AAEL005641), a transferrin (AAEL0015458), two CLIP-domain serine proteases (CLIPB46 AAEL005431, CLIPB37 AAEL005093), a peptidoglycan recognition protein (PGRPS1, AAEL009474), a thioester-containing protein (TEP20, AAEL001794), and a conserved hypothetical protein that was down-regulated (CHP, AAEL003467). The values shown are the means ($\pm$ SEM) of three different qRT-PCR experiments with independent samples, at 2 and 15 days post eclosion.



these hypotheses to be tested. Cecropins are also known to have a major inhibitory effect on *Plasmodium* development (19, 20). Furthermore, the orthologs of *Rel2*, *TEP20* (gene identifier AAEL001794) (7), and *LRIM1* (AAEL012086), all up-regulated in the presence of wMelPop, have been shown by knockdown studies in *Anopheles* to regulate the intensity of *Plasmodium* infection (21–26), as has mosquito midgut microbiota by means of immune stimulation (27). wMelPop-transfected *Anopheles* species should be tested for their ability to inhibit transmission of *Plasmodium* in order to evaluate this malaria control strategy.

Around 120 million people across the tropics are infected with the filarial nematode species that cause lymphatic filariasis, a leading global cause of disability. *Ae. aegypti* is not a natural vector of the disease; however, other *Aedes* species are primary regional vectors, such as *Ae. polynesiensis* in the South Pacific region. The need for novel control methods for *Ae. polynesiensis* has been previously highlighted (28). Sustained efforts to eliminate filariasis in the region by mass drug administration alone have failed; this

is thought to be because *Ae. polynesiensis* is such an efficient vector of *Wuchereria bancrofti*, the main causative agent of human lymphatic filariasis, at low microfilarial densities. Another attractive target is *Culex quinquefasciatus*, the major urban vector of *W. bancrofti* across the tropics. Once stable wMelPop infections are created, they would need to be challenged directly with *W. bancrofti*, because it remains possible that the effects observed are species-specific, but the prospect of a self-spreading transmission-reduction system is attractive, particularly given that it is likely to be especially effective when microfilarial densities in the human population are low.

The data reported here suggest wMelPop may represent a valuable tool in the global effort to eradicate lymphatic filariasis and possibly for the control of other mosquito-borne parasites. The combination of direct inhibitory effects on filariae in the mosquito, together with life-span shortening, could have a powerful overall impact in reducing disease transmission. The ability of wMelPop, in common with many other *Wolbachia* strains, to drive itself through

populations using CI makes this a very attractive strategy.

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29. This study was supported by the Wellcome Trust, European Union's Framework Programme F7 award 223241, European Molecular Biology Organization, and University of Oxford John Fell Fund. We thank S. O'Neill for providing us with the wMelPop-infected and cured *Ae. aegypti* lines PGYP1 and PGYP1.tet; to E. Devaney, for providing *Brugia pahangi* and RefTM *Ae. aegypti*; to M. Povelones, for assistance identifying *Ae. aegypti* LRIM genes; and to A. Brown for comments on the manuscript. Microarray data have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus database, accession GSE117469.

Fig. 3. wMelPop-infected mosquitoes show a reduction in filarial infection prevalence and intensity. The mean numbers ($\pm$ SEM) of infective third (L3) stage *Brugia pahangi* larvae are shown at 10 to 13 days post microfilarial challenge in *Ae. aegypti* wMelPop-infected (Ae_Pop) versus uninfected (Ae_Tet) lines, that are filaria-susceptible after backcrossing with the RefTM strain. Four independent challenge experiments are shown with microfilarial densities (microfilariae per microliter blood) of 11 (experiments A and B), 13 (C), and 23 (D). Values above bars show the prevalence of filarial infection as a proportion of mosquitoes that contained at least one L3 *Brugia* larva versus the total number of mosquitoes dissected in each category. Differences were significant at $*P < 0.01$ or $**P < 0.001$ (Mann-Whitney U test).

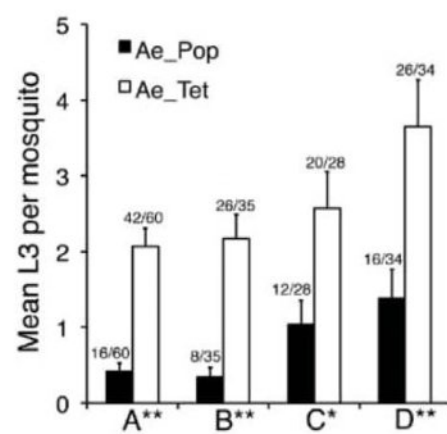
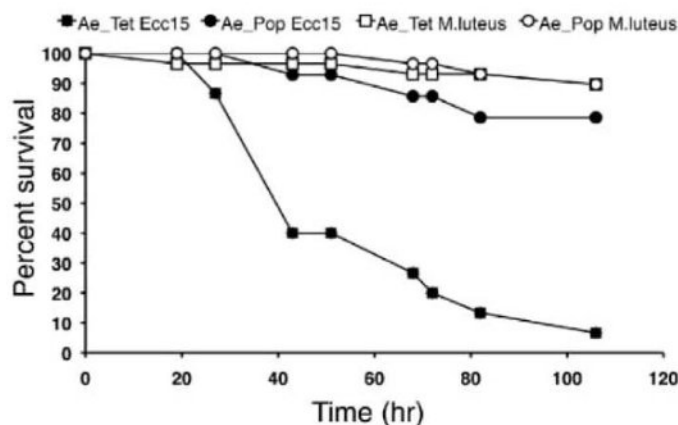


Fig. 4. Increased resistance to a pathogenic bacterium in wMelPop-infected mosquitoes. The daily survival rates (%) of *Ae. aegypti* carrying wMelPop (Ae_Pop) were compared with the *Wolbachia*-free line (Ae_Tet) after infection with *E. carotovora* 15 (Ecc15), a pathogenic Gram-negative bacterium. The horizontal axis represents the incubation time after infection in hours. Control infections with the Gram-positive *M. luteus* demonstrate that the early death or protection effects are not due to septic wounding alone.



Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/134/DC1
Materials and Methods
Tables S1 and S2
References

10 June 2009; accepted 21 August 2009
10.1126/science.1177531

Genetic Discontinuity Between Local Hunter-Gatherers and Central Europe's First Farmers

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After the domestication of animals and crops in the Near East some 11,000 years ago, farming had reached much of central Europe by 7500 years before the present. The extent to which these early European farmers were immigrants or descendants of resident hunter-gatherers who had adopted farming has been widely debated. We compared new mitochondrial DNA (mtDNA) sequences from late European hunter-gatherer skeletons with those from early farmers and from modern Europeans. We find large genetic differences between all three groups that cannot be explained by population continuity alone. Most (82%) of the ancient hunter-gatherers share mtDNA types that are relatively rare in central Europeans today. Together, these analyses provide persuasive evidence that the first farmers were not the descendants of local hunter-gatherers but immigrated into central Europe at the onset of the Neolithic.

Europe has witnessed several changes in archaeological cultures since anatomically modern humans displaced the Neandertal population 30,000 to 40,000 years ago (1, 2). Palaeolithic hunter-gatherers survived the Last Glacial Maximum (LGM) about 25,000 years ago in southern and eastern refugia (3) and re-settled central Europe after the retreat of the ice sheets. With the end of the Ice Age at ~9600 B.C.E., their Mesolithic descendants or successors had recolonized large parts of the deglaciated northern latitudes (4, 5). From around 6400 B.C.E., the hunter-gatherer way of life gave way to farming cultures in a transition known as the Ne-

olithic Revolution (6). The extent to which this important cultural transition was mediated by the arrival of new peoples, and the degree of Mesolithic and early Neolithic ancestry in Europeans today, have been debated for more than a century (7–10). To address these questions directly, we obtained mitochondrial DNA (mtDNA) types from 22 central and northern European post-LGM hunter-gatherer skeletal remains (Fig. 1) and compared 20 of these (those for which full sequence information was available) to homologous mtDNA sequences from 25 early farmers (11, 12) and 484 modern Europeans from the same geographic region (13). Our ancient sample spans a period from

circa (ca.) 13,400 to 2300 B.C.E. and includes bones from Hohler Fels in the Ach valley (Late Upper Paleolithic) and Hohlenstein-Stadel in the Lone valley (Mesolithic). Extensive precautions were taken to ensure sequence authenticity (14), including extracting independent samples from different skeletal locations of the same individuals and examining remains only from high latitudes or cave sites with good biomolecular preservation.

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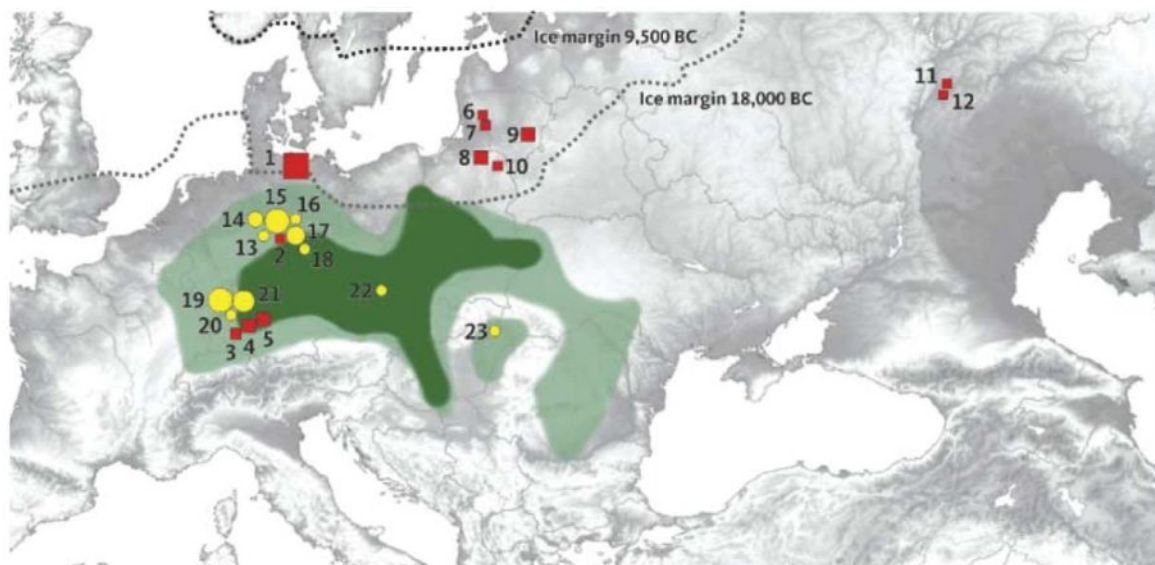
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Fig. 1. mtDNA types from prehistoric samples of hunter-gatherers and farmers. The green shading represents the first farming areas [dark green: early LBK, 5650 to 5400 calibrated years B.C.E. (calBC); light green: LBK, 5400 to 4900 calBC] in central Europe, based on archaeological finds, whereas squares represent successfully analyzed Late Palaeolithic, Mesolithic, and Ceramist hunter-gatherers dating from 13,400 to 2300 B.C.E. The term "Neolithic" is sometimes applied to the Eastern European Ceramist culture because of their use of pottery, but this does not imply a farming economy (21). Previously analyzed (11, 12) LBK farming sites are marked with circles for comparison. The area of each square or circle is proportional to the number of individuals successfully investigated. In red are labeled archaeological sites with one or more U4/U5 individuals; in yellow, sites with other mtDNA types, highlighting the specificity of U types in the prehistoric hunter-gatherers.



The sites are as follows: 1, Ostorf; 2, Bad Dürrenberg; 3, Falkensteiner Höhle; 4, Hohler Fels; 5, Hohlenstein-Stadel; 6, Donkainis; 7, Spiginas; 8, Dudka; 9, Kretuonas; 10, Drestwo; 11, Chekalino; 12, Lebyazhinka; 13, Unseburg; 14, Unterwiederstedt; 15, Derenburg/Meerenstieg; 16, Eilsleben; 17, Halberstadt; 18, Seehausen; 19, Flomborn; 20, Vaihingen an der Enz; 21, Schwetzingen; 22, Asparn/Schletz; 23, Ecsegfalva.

The sites are as follows: 1, Ostorf; 2, Bad Dürrenberg; 3, Falkensteiner Höhle; 4, Hohler Fels; 5, Hohlenstein-Stadel; 6, Donkainis; 7, Spiginas; 8, Dudka; 9, Kretuonas; 10, Drestwo; 11, Chekalino; 12, Lebyazhinka; 13, Unseburg; 14, Unterwiederstedt; 15, Derenburg/Meerenstieg; 16, Eilsleben; 17, Halberstadt; 18, Seehausen; 19, Flomborn; 20, Vaihingen an der Enz; 21, Schwetzingen; 22, Asparn/Schletz; 23, Ecsegfalva.

An analysis of the molecular variance (15) showed that our early farmers and hunter-gatherers were from two well-differentiated populations; the among-populations proportion of genetic variation (F_{ST}) = 0.163, $P < 10^{-6}$. To put this value into perspective, we compared a range of modern human populations, randomly sampling 20 individuals from each. The maximum F_{ST} value in all comparisons among eight modern European samples was 0.0327, and among 13 modern European, Middle Eastern, Indian, Chinese, Papua New Guinean, and Australian samples it was 0.133 (14). We also found that our modern European sample was significantly different from the early farmer ($F_{ST} = 0.0580$, $P = 10^{-5}$) and hunter-gatherer ($F_{ST} = 0.0858$, $P < 10^{-6}$) samples. To test whether these genetic differences can be explained under the null hypothesis of population continuity alone, we performed coalescent simulations across a wide range of ancestral population size combinations. We conservatively assumed a modern female effective population size of $N_0 = 12,000,000$ (one-tenth of the current female population size of central and northern Europe) and two periods of exponential growth: the first after the Upper Paleolithic colonization of Europe 45,000 years ago of female effective population size N_{UP} , sampled from an ancestral African population of constant female effective size $N_A = 5000$; and the second after the Neolithic transition in central Europe 7500 years ago of effective population size N_N . We sampled sequences from each simulation according to the numbers (hunter-gatherer $n = 20$, early farmer $n = 25$, modern $n = 484$) and dates (Table 1) of the sequences presented here and found the proportion of simulated F_{ST} values that were greater than those observed ($P_{S>O}$) (14). By exploring all combinations of 100 values for N_{UP} (ranging from 10 to 5000) and 100 values for N_N (ranging from 1000 to 100,000), we found that the maximum $P_{S>O}$ value between hunter-gatherers and early farmers was 0.022 (for $N_{UP} = 4960$ and $N_N = 1000$), and the maximum $P_{S>O}$ value between hunter-gatherers and modern central Europeans was 0.028 (for $N_{UP} = 3560$ and $N_N = 1000$). Most $P_{S>O}$ values were considerably lower (Fig. 2). These results allow us to reject direct continuity between hunter-gatherers and early farmers, and between hunter-gatherers and modern Europeans.

When we considered continuity between early farmers and modern Europeans, we did identify ancestral population size combinations where $P_{S>O} > 0.05$ (black shaded area in Fig. 2C). Thus, there are demographic conditions under which the observed genetic differences between early European farmers and modern Europeans can be explained by assuming population continuity. Those conditions include assuming $N_N < 3000$, an effective female population size that may be considered implausibly low and is certainly lower than the current archaeological census estimates of 124,000 (16). However, we note that (i) ancestral population sizes are notoriously difficult to estimate from

archaeological data, and (ii) the relationship between effective and census population size is dependent on unknown factors, including mating systems and population substructure.

Most modern European mtDNA lineages can be assigned to one of the following clades or haplogroups: H, V, U (including K), J, or T, all deriving from clade R; or I, W, or X, the descendants of clade N. Although some subclades, such as U5, are fairly specific to Europe, most are shared with adjacent areas of Asia and North Africa and are of uncertain antiquity in Europe. We are therefore cautious about treating specific clades as markers of particular past population groups or demographic episodes (17). Nonetheless, it is intriguing to note that 82% of our 22 hunter-gatherer individuals carried clade U (14 U5, 2 U4, and 2 unspecified U types; Table 1). A high incidence of U types (particularly those belonging to the U5 subclade) in Stone Age Europeans has been inferred from modern mtDNA

(7), but the frequencies found here are surprisingly high. Europeans today have moderate frequencies of U5 types, ranging from about 1 to 5% along the Mediterranean coastline to 5 to 7% in most core European areas, and rising to 10 to 20% in northeastern European Uralic speakers, with a maximum of over 40% in the Scandinavian Saami. U4 types show frequencies between 1 and 5% in most parts of Europe, with Western Europe at the lower end of this range and northeastern Europe and central Asia showing percentages in excess of 7% (13).

The diversity among the hunter-gatherer U types presented here, together with their continued presence over 11 millennia, and the fact that U5 is rare outside Europe, raises the possibility that U types were common by the time of the post-LGM repopulation of central Europe, which started around 23,000 years ago (3). In a previous study, we showed that the early farmers of central Europe carried mainly N1a, but also H, HV, J, K,

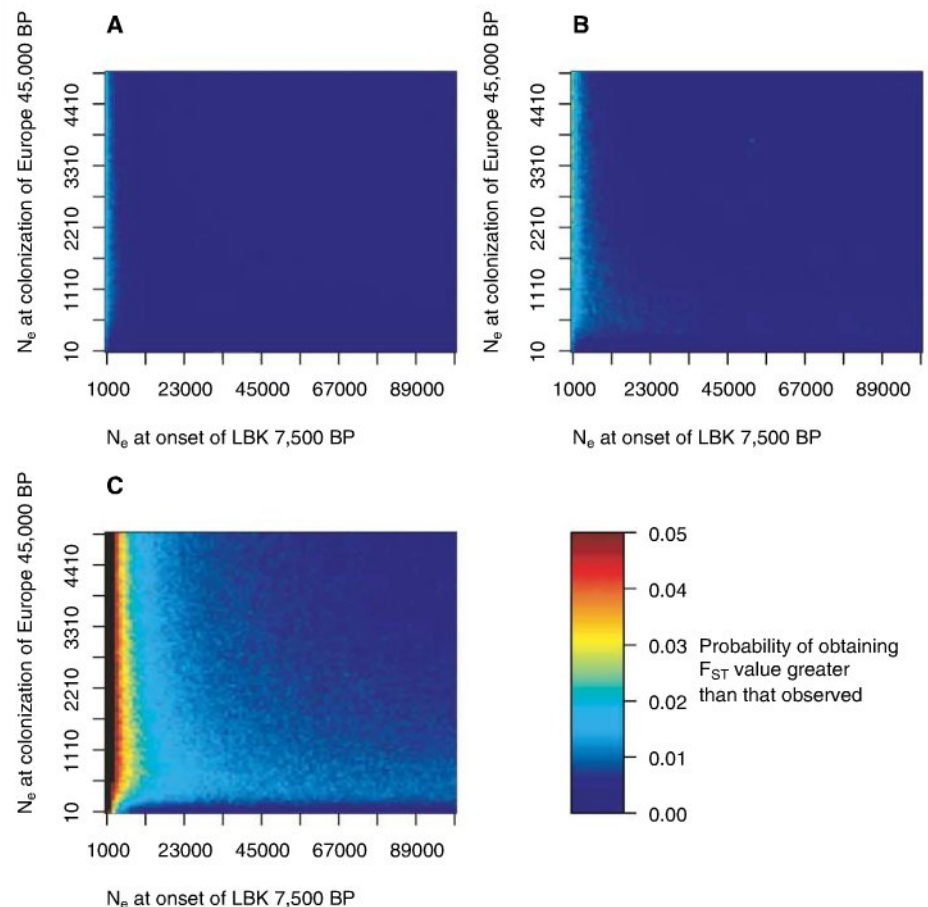


Fig. 2. Probabilities of obtaining observed genetic differences, as measured by F_{ST} , between (A) hunter-gatherers and LBK early farmers, (B) hunter-gatherers and modern Europeans, and (C) LBK early farmers and modern Europeans, across a range of assumed ancestral population size combinations. Two phases of exponential growth were considered, the first after the initial colonization of Europe 45,000 years ago, of assumed effective female population size N_{UP} (y axis), and ending when farming began in central Europe 7500 years ago, when the assumed effective female population size was N_N (x axis); and the second leading up to the present, when the assumed effective female population size is 12 million. The initial colonizers of Europe were sampled from a constant ancestral African population of 5000 effective females. The F_{ST} values are those observed from the data presented in this study. Black shaded areas indicate probabilities >0.05 .

T, V, and U3 types (11, 12). We found no U5 or U4 types in that early farmer sample. Conversely, no N1a or H types were observed in our hunter-gatherer sample, confirming the genetic distinctiveness of these two ancient population samples. This is particularly surprising as there is clear evidence for some continuity in the material culture between the central European Mesolithic and the earliest settlements of the Neolithic Linearbandkeramik culture (LBK) (18). Thus, it seems that despite the exchange of stone artifacts, genetic exchange between both groups, at least on the female side, was initially limited. The only exception is the site Ostorf (northern Germany), where two individuals carried haplogroup T2, which is also found in our LBK sample. We are cautious about interpreting this as a signature of local admixture (17), particularly because the hunter-gatherer and early farmer T2 types belong

to different sublineages, but it is notable that Ostorf is culturally a Mesolithic enclave surrounded by Neolithic funnel-beaker farmers and is the only hunter-gatherer site where any non-U mtDNA types were observed (Table 1). Further sampling from such local contexts should shed light on the details of Mesolithic-Neolithic interactions after the arrival of farming. We note that any genetic exchange between hunter-gatherers and early farmers at this site would reduce the overall genetic differentiation between the two groups, so inclusion of this site has, if anything, a conservative effect on our conclusions regarding continuity.

Taken together, our results indicate that the transition to farming in central Europe was accompanied by a substantial influx of people from outside the region who, at least initially, did not mix significantly with the resident female hunter-

gatherers. We accept that alternative, more complex demographic scenarios, such as strong local population structure and high group extinction and fission rates, might also explain our data. However, the ubiquity of U types in our hunter-gatherer samples is inconsistent with extensive population structuring and indicates that the demographic processes that shaped the observed patterns of genetic variation extend beyond the local scale.

The extent to which modern Europeans are descended from incoming farmers, their hunter-gatherer forerunners, or later incoming groups remains unresolved. The predominant mtDNA types found in the ancient samples considered in this study are found in modern Europeans, but at considerably lower frequencies, suggesting that the diversity observed today cannot be explained by admixture between hunter-gatherers and early

Table 1. Stone Age individuals and their mtDNA results. A, DNA of the archaeologists available for comparison; D, diagenetical analysis; M, multiple extractions and number of these; C, clones of the hypervariable segment 1 and number of these; N, positive amplification of nuclear DNA; Rf, restriction fragment length polymorphism analysis; SNP, single-nucleotide polymorphisms from the coding region of mtDNA obtained by means of multiplex

amplification; BP, before the present; ca., circa. The mtDNA was sequenced from nucleotide position (np) 15997 to np 16409. mtDNA positions are numbered according to the revised Cambridge reference sequence (22), minus 16,000. Fourteen individuals did not yield results (table S1), whereas for two individuals the mtDNA sequences were not determined (n.d.) and thus not considered in the AMOVA analysis and simulations.

Country	Site, skeleton	Basis of dating*	Dating calBC*	Analyses	mtDNA sequence	Clade
Lithuania	Spiginas 4	GIN-5571: 7470 ± 60 BP	ca. 6350 calBC	A, M3, C109, Q, Rf	356c	U4
	Donkainis 1	Cultural context	Mesolithic	A, D, M4, C79, N, Rf, SNP	192t 270t	U5b2
	Kretuonas 3	OxA-5926: 5580 ± 65 BP	ca. 4450 calBC	A, M4, C72, N, Rf, SNP	192t 270t	U5b2
	Kretuonas 1	OxA-5935: 5350 ± 130 BP	ca. 4200 calBC	A, M5, C56, N, Rf, SNP	192t 270t	U5b2
Poland	Dudka 2	¹⁴ C date on charcoal	ca. 3650 calBC	A, M3, C80, N, Rf	189c 270t	U5b1
	Dudka 3	Cultural context	4000-3000 calBC	A, M3, C127, Q, Rf	189c 265 g 270t	U5b1
	Drestwo 2	Ua-13085: 3805 ± 70 BP	ca. 2250 calBC	D, M4, C102, N, Rf	192t 256t 270t	U5a
Russia	Chekalino IVa	¹⁴ C date on shell	ca. 7800 calBC	A, D, M2, C83, Rf	192t 256t 270t 294t	U5a
	Chekalino IVb					
Germany	Lebyazhinka IV	¹⁴ C date on shell and cultural context	8000–7000 calBC	A, D, M2, C60, Rf	192t 241a/c 256t 270t 399 g	U5a1
	Bad Dürrenberg 2	OxA-3136: 7930 ± 90 BP	ca. 6850 calBC	A, D, M2, C 119, Rf	356c	U4
	Hohlenstein-Stadel, 5830a	ETH-5732: 7835 ± 80 BP	ca. 6700 calBC	M1, SNP	114a 192t 256t 294t 311c	U5a1
	Hohlenstein-Stadel, 5830b	ETH-5732: 7835 ± 80 BP	ca. 6700 calBC	M1, SNP	192t 270t	U5b2
	Hohler Fels, 49 Ib1 66	¹⁴ C dates on bone (H 5312-4907: 12,770 ± 110 BP; H 5119-4601: 13,085 ± 95 BP) and cultural context	Magdalenian ca. 13,400 calBC	M2, SNP	CR5	U
Germany	Hohler Fels, 10 Ic 405	¹⁴ C dates on bone (H 5312-4907: 12,770 ± 110 BP; H 5119-4601: 13,085 ± 95 BP) and cultural context	Magdalenian ca. 13,400 calBC	M2, SNP	n.d.	U
	Falkensteiner Höhle, FH	ETH-7615: 8185 ± 80 BP	ca. 7200 calBC	M2, SNP	n.d.	U5b2
	Ostorf SK28a	¹⁴ C dates and context	ca. 3200 calBC	A, M2, C18	224c 311c	K
	Ostorf SK8d	¹⁴ C dates and context	ca. 3200 calBC	A, M2, C16	270t	U5
	Ostorf SK35	¹⁴ C dates and context	ca. 3100 calBC	A, M2	270t	U5
	Ostorf SK12a	¹⁴ C dates and context	ca. 3000 calBC	A, M2	093y 126c 153a 294t	T2e
	Ostorf SK45a	¹⁴ C dates and context	ca. 3000 calBC	A, M2, C16	069t 126c	J
	Ostorf SK18	¹⁴ C dates and context	ca. 3000 calBC	A, M4	093c 126c 153a 294t	T2e
	Ostorf SK19	¹⁴ C dates and context	ca. 2950 calBC	A, M3	168t 192t 256t 270t 302 g	U5a

*Radiocarbon dates with laboratory numbers refer to direct dates of the skeleton and were calibrated with the program CalPal (23) on the basis of Intcal04. Corrections of reservoir effects were applied where identified.

farmers alone. If this is the case, then subsequent dilution through migration and admixture, after the arrival of the first farmers, would need to be invoked, implying multiple episodes of population turnover, which are not necessarily observable in the archaeological record. This, in turn, would mean that the classic model of European ancestry components (contrasting hunter-gatherers with early Neolithic farming pioneers) requires revision.

The geographic origin of the demographic processes that brought the early farmer mtDNA types to central Europe now becomes a major question. On the one hand, all of the early farmer remains analyzed here are associated with the LBK culture of central Europe. Based on ceramic typology, the LBK culture is thought to have originated in present-day western Hungary and southwestern Slovakia, with a possible predecessor in the southeast European Starčevo-Kris culture (19, 20). These cultural source locations may provide the most plausible origins or routes for the geographic spread of the early farmers, considering that the LBK was the first major farming culture in central and northern Europe and is archaeologically attested to have disseminated over five centuries and covered nearly a million square kilometers. Alternatively, the farmers' mtDNA types may have an origin closer to the Neolithic core zone in southwestern Asia. Further ancient DNA analysis of early farmer samples from southeastern Europe and Anatolia will be required to resolve this question.

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24. The authors are grateful to W. Guminski (Institute of Archeology and Ethnology, Polish Academy of Sciences, Warsaw, Poland), J. Siemaszko (Suwalki Province Museum, Suwalki, Poland), A. Khokhlov (Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow oblast, Russia), H. Meller and M. Porz (Landesamt für Archäologie Sachsen-Anhalt, Halle/Saale, Germany), and L. P. Louwe Kooijmans and L. Smits (Faculty of Archeology, Leiden University, Leiden, Netherlands) for providing them with the archaeological samples. The authors are indebted to R. Villes, A. Zimmermann, and J. Lüning for comments and to D. Kasperaviciute for Lithuanian sequences. We also thank M. Forster for editorial comments. Research grants were provided by the Bundesministerium für Bildung und Forschung, the Deutsche Forschungsgemeinschaft, and the Estonian Science Foundation (grant no. 6040 to K.T.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/1176869/DC1

Materials and Methods

Fig. S1

Tables S1 to S6

References

27 May 2009; accepted 21 August 2009

Published online 3 September 2009;

10.1126/science.1176869

Include this information when citing this paper.

Ribosomal Protein S6 Kinase 1 Signaling Regulates Mammalian Life Span

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Caloric restriction (CR) protects against aging and disease, but the mechanisms by which this affects mammalian life span are unclear. We show in mice that deletion of ribosomal S6 protein kinase 1 (S6K1), a component of the nutrient-responsive mTOR (mammalian target of rapamycin) signaling pathway, led to increased life span and resistance to age-related pathologies, such as bone, immune, and motor dysfunction and loss of insulin sensitivity. Deletion of *S6K1* induced gene expression patterns similar to those seen in CR or with pharmacological activation of adenosine monophosphate (AMP)–activated protein kinase (AMPK), a conserved regulator of the metabolic response to CR. Our results demonstrate that S6K1 influences healthy mammalian life span and suggest that therapeutic manipulation of S6K1 and AMPK might mimic CR and could provide broad protection against diseases of aging.

Genetic studies in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* implicate several mechanisms in the regulation of life span. These include the insulin and insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway and the mammalian target of rapamycin (mTOR) pathway,

which both activate the downstream effector ribosomal protein S6 kinase 1 (S6K1) (1, 2). Although the role of these pathways in mammalian aging is less clear, there is mounting evidence that IIS regulates life span in mice (3). Global deletion of one allele of the IGF-1 receptor (*Igf1r*), adipose-specific deletion of the insulin receptor (*Insr*),

global deletion of insulin receptor substrate protein 1 (*Irs1*), or neuron-specific deletion of *Irs2*, all increase mouse life span (4). Life-span-extending mutations in the somatotrophic axis also appear to work through attenuated IIS (5). *Igf1r* has also been implicated as a modulator of human longevity (6). However, the action of downstream effectors of IIS or mTOR signaling in mammalian longevity is not fully understood.

S6K1 transduces anabolic signals that indicate nutritional status to regulate cell size and

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growth and metabolism through various mechanisms (5). These include effects on the translational machinery and on cellular energy levels through the activity of adenosine monophosphate (AMP)-activated protein kinase (AMPK) (6, 7). Furthermore, S6K1 serine phosphorylates IRS1 and IRS2, which decreases insulin signaling (5). Given the key role of S6K1 in IIS and mTOR signaling and the regulation of aging in lower organisms by mTOR, S6K, and their downstream effectors (2), we used log-rank testing to evaluate differences in life span of wild-type (WT) and *S6K1*^{-/-} littermate mice on a C57BL/6 background (8, 9). Data for both sexes combined showed median life-span in *S6K1*^{-/-} mice increased by 80 days (from 862 to 942 days) or 9% relative to that of WT mice ($\chi^2 = 10.52$, $P < 0.001$) (Fig. 1A and Table 1). Maximum life span (mean life span of the oldest 10% within a cohort) was also increased (1077 ± 16 and 1175 ± 24 days, $P < 0.01$ for WT and *S6K1*^{-/-} mice, respectively). Analysis of each sex separately showed that median life span in female *S6K1*^{-/-} mice was increased by 153 days (from 829 to 982 days) or 19% relative to that of WT mice ($\chi^2 = 11.07$, $P < 0.001$) (Fig. 1B and Table 1). Female maximum life-span was also increased (Table 1). In contrast, deletion of *S6K1* in male mice had no effect on median ($\chi^2 = 0.34$, $P > 0.05$) (Fig. 1C and Table 1) or maximum life span (Table 1). Similar gender effects on life span have been reported in other long-lived IIS mouse mutants (8, 10). Cox

regression analysis of pooled male and female life-span data revealed no effect of recruitment date, parental identity, or gender, but that of genotype was significant (table S1). Therefore, deletion of *S6K1* increases longevity in female mice.

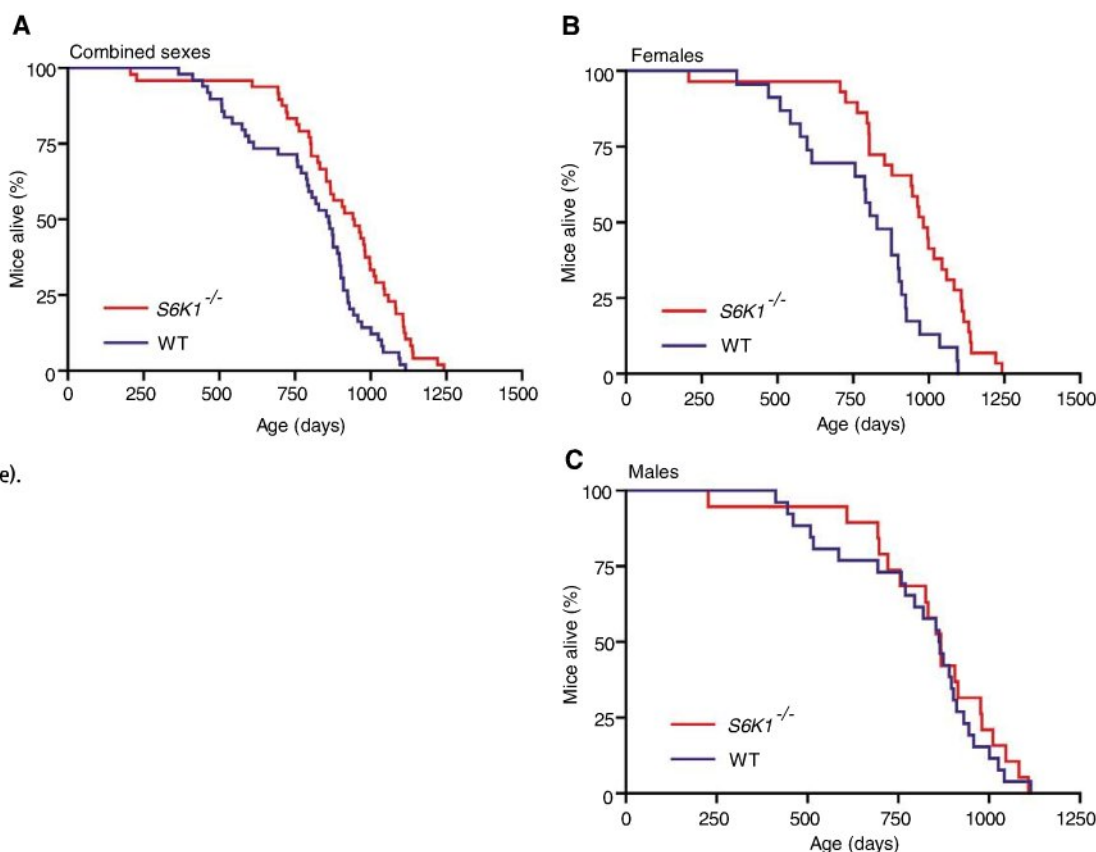
Female *S6K1*^{-/-} mice also showed improvements in a number of age-sensitive biomarkers of aging. In forced motor activity on a rotating rod (rotarod) assays to assess motor and neurological function, 600-day-old female *S6K1*^{-/-} mice performed better than WT littermates (Fig. 2A). Performance in open-field testing to analyze general activity and exploratory drive were also enhanced (Fig. 2, B and C). An increase in abundance of memory T cells and a reduced number of naïve T cells are seen in mice with age, and the extent of these changes may be correlated with longevity (11). Female *S6K1*^{-/-} mice at 600 days of age had significantly fewer memory and more naïve T cells than did WT mice (Fig. 2D), although male mice also displayed this phenotype (fig. S1A). Micro-computed tomography scanning of tibia from 600-day-old female *S6K1*^{-/-} mice revealed attenuation of the normal age-dependent loss of cancellous bone volume seen in C57BL/6 mice (12) (Fig. 2, E and F). However, there was no difference in the incidence of macroscopic tumors in *S6K1*^{-/-} and WT animals [8% (4 out of 48) for *S6K1*^{-/-} and 8% (4 out of 49) for WT mice, respectively].

Young, male *S6K1*^{-/-} mice fed a high-fat diet (13) display increased insulin sensitivity and re-

duced adiposity relative to those of WT mice, phenotypes also seen in WT mice under caloric restriction (CR), an evolutionarily conserved environmental manipulation that extends life span (14). Insulin sensitivity (assessed by the updated homeostasis model, HOMA2) was significantly greater in 600-day-old female *S6K1*^{-/-} mice than in WT animals (Fig. 2G), and glucose tolerance was improved (Fig. 2H), in contrast to the impaired glucose tolerance seen in young animals (fig. S1B). Fat mass and plasma leptin levels were lower in old female *S6K1*^{-/-} mice (Fig. 2, I and J), despite increased food intake (fig. S1C). Core temperature and resting metabolic rate (with general linear modeling to account for body-mass differences) were not significantly different (fig. S1, D and E). Although *S6K1*^{-/-} mice were smaller than their littermates throughout their lives (Fig. 2K), endocrinologically they did not resemble long-lived pituitary dwarfs (15), because their total circulating IGF-1, pituitary growth hormone, thyroid-stimulating hormone, and prolactin concentrations were normal (Fig. 2, L and M, and fig. S1, F and G). Male *S6K1*^{-/-} mice at 600 days of age had normal fasting and fed glucose levels (fig. S1, H and I).

We compared the effect of *S6K1* deletion on genome-wide hepatic gene expression in 600-day-old female mice to transcriptional changes induced by long-term CR (16). The 500 gene categories most overrepresented among genes with altered expression in *S6K1*^{-/-} mice showed

Fig. 1. Extended life span of mice with deletion of *S6K1*^{-/-}. **(A)** Kaplan-Meier survival curves for combined male and female wild-type (WT) and *S6K1*^{-/-} mice show a significant (log-rank $\chi^2 = 10.52$, $P < 0.001$) life-span extension in *S6K1*^{-/-} mice ($n = 49$ for WT mice and $n = 48$ for *S6K1*^{-/-} mice). **(B)** Life-span extension was observed in female *S6K1*^{-/-} mice ($\chi^2 = 11.07$, $P < 0.001$; $n = 23$ for WT mice and $n = 29$ for *S6K1*^{-/-} mice). **(C)** No significant increase in life span in *S6K1*^{-/-} mice ($\chi^2 = 0.34$, $P > 0.05$; $n = 26$ for WT mice and $n = 19$ for *S6K1*^{-/-} mice).



a highly significant overlap with categories over-represented among CR-regulated genes ($P = 3.25 \times 10^{-42}$ and $P = 1.15 \times 10^{-19}$ for up- and down-regulated categories, respectively, Fisher's exact test) (fig. S2A). Hepatic transcript profiles in long-lived *Irs1*^{-/-} mice (8) were also similar ($P = 1.60 \times 10^{-21}$ and $P = 8.61 \times 10^{-20}$ for up- and down-regulated categories, respectively) (fig. S2B). Furthermore, we observed significant correlations in the directions of transcriptional

changes associated with highly significant functional categories ($P < 10^{-4}$, two-tailed) in both comparisons (fig. S2, A and B). This is consistent with the existence of common mechanisms underlying the effects of S6K1, CR, and IIS on aging.

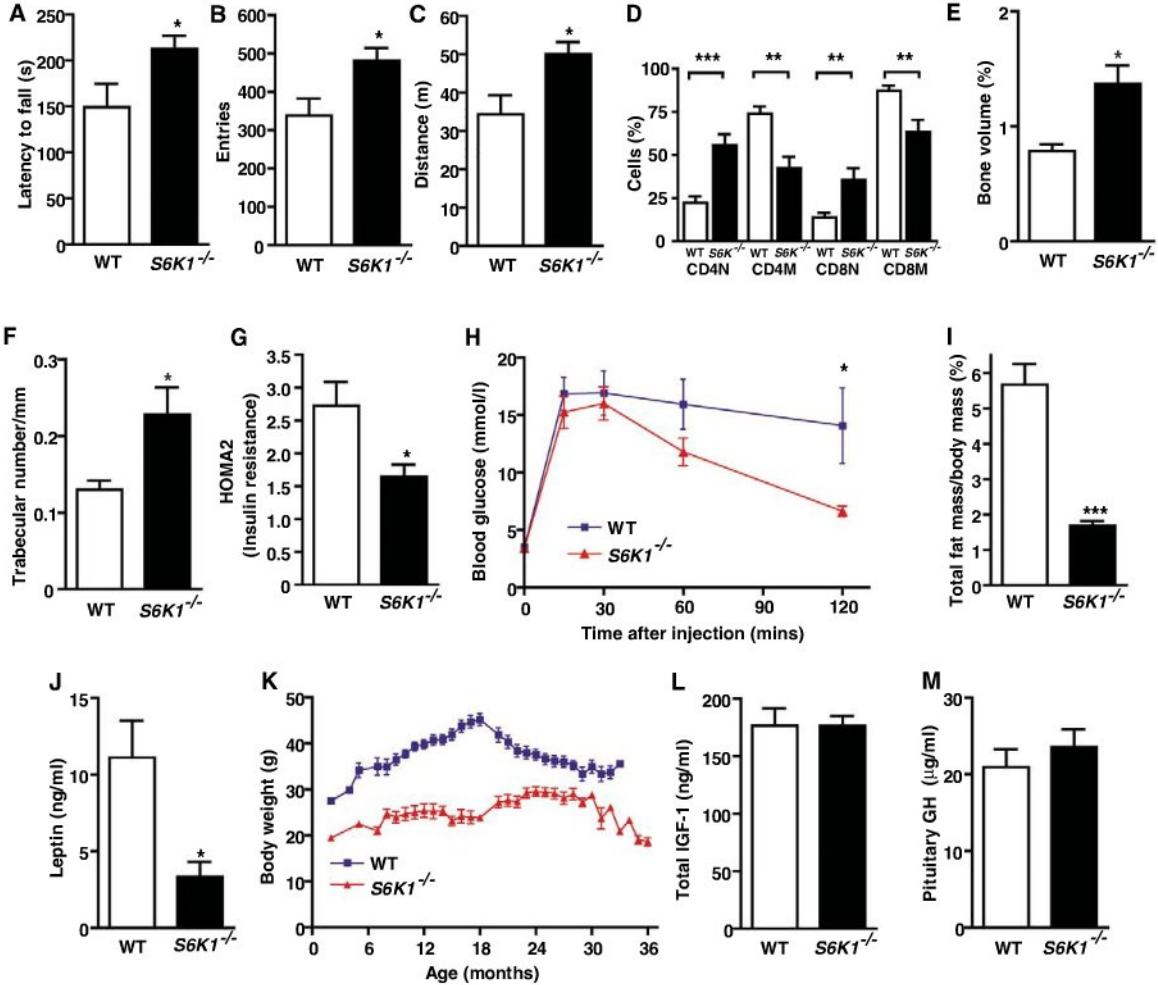
We examined transcription of individual genes in liver, skeletal muscle, and white adipose tissue (WAT) in 600-day-old female *S6K1*^{-/-} and WT mice, looking for genes previously associated

with longevity (tables S2, A and B, S3, A and B, and S4, A and B). Significant cross talk exists between peroxisome proliferator-activated receptor (PPAR)- γ , coactivator 1 α (PGC-1 α), AMPK, and nicotinamide adenine dinucleotide (oxidized form)-dependent deacetylase sirtuin-1 (SIRT1) signaling, which may be critical to cellular energy metabolism and perhaps aging (17). Increased expression of genes associated with these pathways was observed in liver (*Pparg1a*, *Pparg1b*, *Foxo1*, *Foxo3a*, *Cpt1b*, *Pdk4*, *Glut1*, and *Cyc*) and muscle (*Pparg1a*, *Ppara*, *Foxo1*, *Foxo3a*, *Pdk4*, *Glut1*, *Sirt1*, and *Ucp3*) of *S6K1*^{-/-} mice. Adipose tissue is a key tissue in longevity assurance in *C. elegans*, *D. melanogaster*, and mice (18). In WAT of *S6K1*^{-/-} mice, fewer PGC-1 α -regulated genes (*Foxo3a*, *Pdk4*, *Nampt*, and *Angptl4*) showed increased expression compared with changes seen in liver and muscle, but there was also increased expression of the $\alpha 2$ catalytic and $\beta 1$ regulatory subunits of AMPK (log 2 fold change = 1.7, $P = 2.88 \times 10^{-6}$ and 1.2, $P = 4.95 \times 10^{-5}$, respectively, Cyber-T analysis). AMPK activity is increased in WAT, muscle, and liver of *S6K1*^{-/-} mice (7). Moreover, comparison of gene expression patterns in muscle of *S6K1*^{-/-} mice with those of mice treated with the AMPK activator aminoimidazole carboxamide ribo-

Table 1. Comparative survival characteristics of *S6K1*^{-/-} and WT mice. Oldest (youngest) 10% are the mean life span of the longest (or shortest) living 10% of animals within a genotype. Values are reported $\pm$ SEM, where appropriate.

Genotype	Life span (days)					n
	Median	Mean	Min–Max	Oldest 10%	Youngest 10%	
Combined sex						
WT	862	796 ± 28	365–1115	1077 ± 16	431 ± 19	49
<i>S6K1</i> ^{−/−}	942	907 ± 30	207–1242	1175 ± 24	487 ± 111	48
Female						
WT	829	789 ± 42	365–1097	1096 ± 2	418 ± 53	23
<i>S6K1</i> ^{−/−}	982	950 ± 38	207–1242	1201 ± 31	546 ± 170	29
Male						
WT	862	801 ± 39	412–1115	1061 ± 28	439 ± 14	26
<i>S6K1</i> ^{−/−}	867	841 ± 47	227–1108	1095 ± 13	418 ± 191	19

Fig. 2. Age-related pathology and physiological characteristics of 600-day-old female *S6K1*^{-/-} mice. (A) *S6K1*^{-/-} mice had improved rotarod performance. (B and C) Increased general activity and exploratory drive was observed in *S6K1*^{-/-} mice. (D) Abundance of memory and naïve T cells in WT and *S6K1*^{-/-} mice. (E and F) Bone volume and trabecular number in WT and *S6K1*^{-/-} mice. (G and H) Insulin sensitivity and glucose tolerance of WT and *S6K1*^{-/-} mice. (I) *S6K1*^{-/-} mice were lean and (J) had reduced plasma leptin levels. (K) Body mass ($P < 0.01$ at all time points), total circulating IGF-1 (L), and pituitary growth hormone (GH) concentrations in WT and *S6K1*^{-/-} mice (M). Values are means $\pm$ SEM. Asterisks indicate statistical difference compared with WT mice by using two-tailed *t* tests, * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$; $n = 6$ to 8 per genotype.



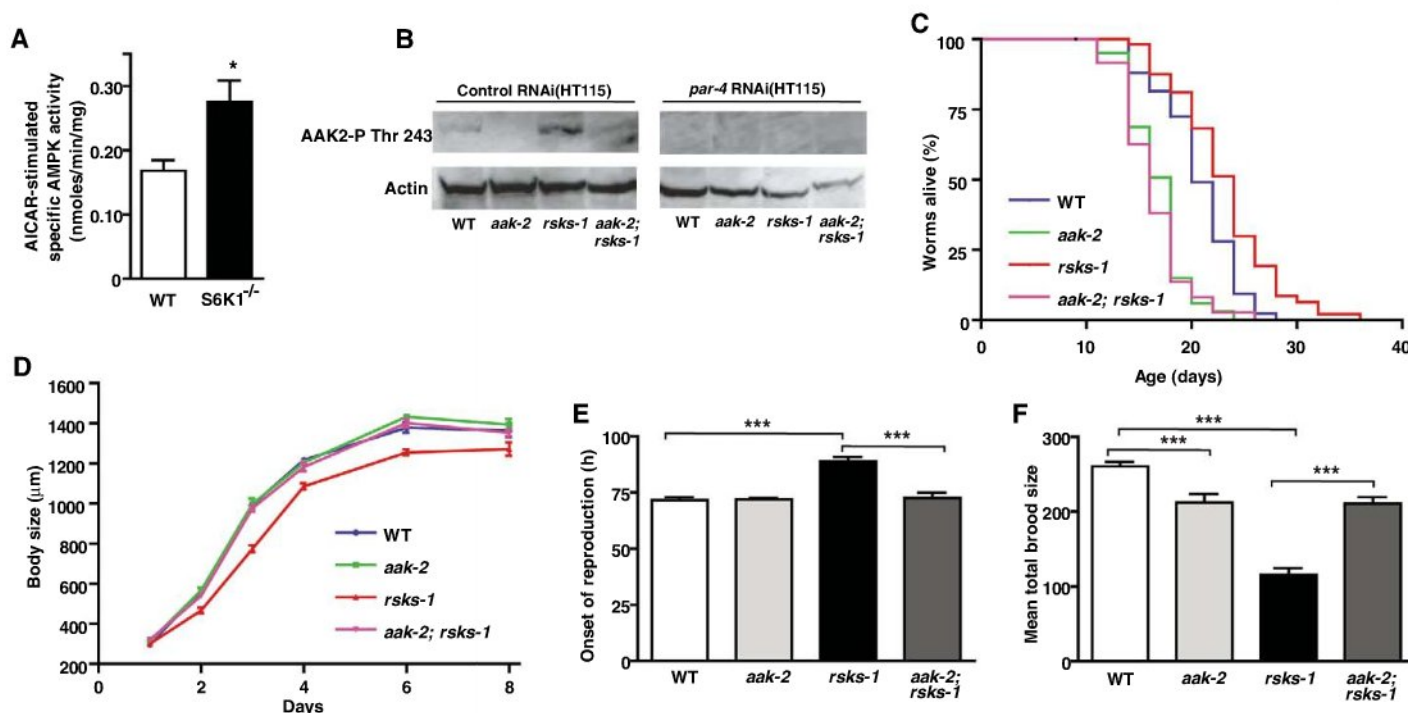


Fig. 3. Enhanced AMPK activation by AICAR of *S6K1*^{-/-} hepatocytes, increased AAK-2 phosphorylation in *rsk-1(ok1255)* mutants, and effects of loss of *aak-2(ok524)* on longevity and physiology. **(A)** AICAR-stimulated AMPK activity in isolated hepatocytes from *S6K1*^{-/-} mice. **(B)** Phosphorylation of AAK-2 Thr²⁴³ in *rsk-1(ok1255)* null mutants subjected, or not, to RNA interference for *par-4*, the worm LKB kinase that effects this phosphorylation. **(C)** Life span of *rsk-1(ok1255)* nulls with mutation of *aak-2(ok524)*. **(D to F)** Body length, onset of reproductive function, and brood-size phenotypes in *rsk-1(ok1255)*

mutants with or without *aak-2(ok524)* mutation. In **(D)**, *rsk-1(ok1255)* is significantly different ($P < 0.001$; one-way ANOVA) from all other groups from day 2 onward, but *rsk-1(ok1255); aak-2* is not significantly different from WT or *aak-2*. **(A)** to **(C)** show data from one representative experiment, and **(D to F)** show combined data from three similar independent experiments. Values **(A and D to F)** are means $\pm$ SEM. In **(A)**, $n = 3$, and in **(D)**, $n > 8$ for each strain and time point. For **(E)** and **(F)**, $n > 20$ for each group. Asterisks indicate statistical differences by using two-tailed t tests, * $P < 0.05$, *** $P < 0.001$.

nucleotide (AICAR) (19) revealed a strong overlap between gene categories that showed increased expression, including those associated with PPAR signaling and lipid metabolism (fig. S2C). We confirmed enhanced AMPK activation by AICAR in isolated hepatocytes from *S6K1*^{-/-} mice (Fig. 3A).

In *C. elegans*, AMPK mediates the effects on life-span of one particular form of CR (20) and perturbing IIS (21, 22), which raises the possibility that the longevity of *S6K1*^{-/-} mice results from increased AMPK activity. To test this, we studied long-lived *C. elegans rsk-1(ok1255)* null mutants, which lack the single worm S6K1 homolog. *rsk-1* mutants showed increased phosphorylation of the worm AMPK catalytic subunit AAK-2 (Fig. 3B), consistent with increased AMPK activity. These findings imply that in worms, as in mice, loss of S6K1 increases AMPK activity. *rsk-1* mutants are long-lived (Fig. 3C, and table S5), with reduced and delayed fecundity and, like *S6K1*^{-/-} mice, reduced body size (Fig. 3, D to F, and table S5), characteristics that could be attributed to reduced nutrient availability or possibly reduced overall translation (23). To test the role of AMPK in mediating the effects of *rsk-1* on longevity, we generated mutants lacking both *rsk-1* and *aak-2*. The *aak-2(ok524)* null allele fully suppressed *rsk-1* mutant longevity (Fig. 3C, and table S5). This effect is likely to be specific,

because several other modes of *C. elegans* longevity are not *aak-2*-dependent (22). Moreover, *aak-2(ok524)* also suppressed the fecundity and body size defects of *rsk-1* mutants (Fig. 3, D to F). This also suggests that these defects do not reflect reduced overall translation; in fact, in muscle cells from growth-deficient *S6K1* null mice, protein synthesis is reportedly not reduced (24). Taken together, these results imply that increased AMPK activity may contribute to the longevity of both *C. elegans* and mice lacking *S6K1*.

Our studies indicate that S6K1 signaling influences mammalian life span and age-related pathology. S6K1 is regulated in response to nutrient and hormonal signals and may thus participate in the response to CR. mTOR and AMPK are amenable to pharmacological intervention (25, 26). It might be possible to develop drug treatments that manipulate S6K1 and AMPK to achieve improved overall health in later life. Indeed, short-term rapamycin treatment reduces adiposity in mice (27), and metformin treatment extends life span in short-lived mice (28). Furthermore, recently it has been demonstrated that rapamycin treatment initiated late in life extends life span in mice (29). Our results suggest that this may occur via inhibition of S6K1, and together, these studies indicate the feasibility of manipulating mTOR/S6K1 signaling in the treatment of aging-related disease.

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Supporting Online Material

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3 June 2009; accepted 24 August 2009
10.1126/science.1177221

Genome-Wide RNAi Screen Identifies Letm1 as a Mitochondrial $\text{Ca}^{2+}/\text{H}^{+}$ Antiporter

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Mitochondria are integral components of cellular calcium (Ca^{2+}) signaling. Calcium stimulates mitochondrial adenosine 5'-triphosphate production, but can also initiate apoptosis. In turn, cytoplasmic Ca^{2+} concentrations are regulated by mitochondria. Although several transporter and ion-channel mechanisms have been measured in mitochondria, the molecules that govern Ca^{2+} movement across the inner mitochondrial membrane are unknown. We searched for genes that regulate mitochondrial Ca^{2+} and H^{+} concentrations using a genome-wide *Drosophila* RNA interference (RNAi) screen. The mammalian homolog of one *Drosophila* gene identified in the screen, *Letm1*, was found to specifically mediate coupled $\text{Ca}^{2+}/\text{H}^{+}$ exchange. RNAi knockdown, overexpression, and liposome reconstitution of the purified Letm1 protein demonstrate that Letm1 is a mitochondrial $\text{Ca}^{2+}/\text{H}^{+}$ antiporter.

Mitochondrial Ca^{2+} uptake across the inner mitochondrial membrane occurs via tightly regulated channels [e.g., MCU/MiCa (1, 2) and transporters (3–6)]. Increases in the concentration of calcium ($[\text{Ca}^{2+}]$)

in the mitochondrial matrix enhance the activities of adenosine 5'-triphosphate (ATP) synthase and enzymes in the tricarboxylic acid cycle (7, 8), but if homeostatic mechanisms fail, high levels of matrix Ca^{2+} induce cell death (9, 10). We set

out to identify the genes that mediate Ca^{2+} flux across the inner mitochondrial membrane.

We conducted a genome-wide, high-throughput RNA interference (RNAi) screen to identify genes that control mitochondrial Ca^{2+} transport. *Drosophila* S2 cells stably expressing mitochondrial-targeted ratiometric pericam were incubated with arrayed double-stranded RNAs (dsRNAs) against each of the ~22,000 *Drosophila* genes (11). Mitochondrial (Mt)-pericam emission due to excitation at 405 nm is sensitive to changes in $[\text{Ca}^{2+}]$, whereas emission in response to excitation at 488 nm independently reports changes in pH (figs. S1 and S2). Through a series of screens discussed in the supporting online material (SOM) (fig. S2 and tables S1 to S3), CG4589, the *Drosophila* homolog of the human gene *Letm1*, was identified as a gene strongly affecting $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$.

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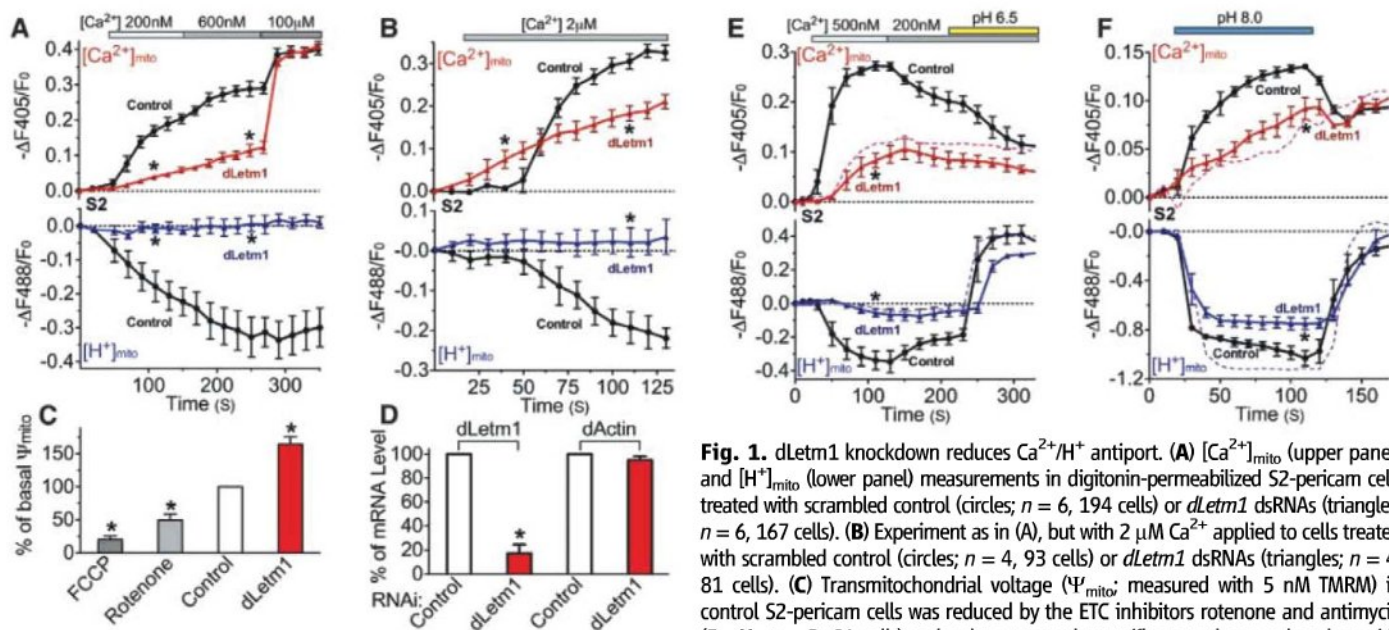


Fig. 1. *dLetm1* knockdown reduces $\text{Ca}^{2+}/\text{H}^{+}$ antiport. **(A)** $[\text{Ca}^{2+}]_{\text{mito}}$ (upper panel) and $[\text{H}^{+}]_{\text{mito}}$ (lower panel) measurements in digitonin-permeabilized S2-pericam cells treated with scrambled control (circles; $n = 6$, 194 cells) or *dLetm1* dsRNAs (triangles; $n = 6$, 167 cells). **(B)** Experiment as in (A), but with 2 μM Ca^{2+} applied to cells treated with scrambled control (circles; $n = 4$, 93 cells) or *dLetm1* dsRNAs (triangles; $n = 4$, 81 cells). **(C)** Transmembrane voltage (Ψ_{mito}) measured with 5 nM TMRM in control S2-pericam cells was reduced by the ETC inhibitors rotenone and antimycin (5 μM , $n = 3$, 81 cells) or by the protonophore trifluoromethoxy carbonyl cyanide

phenylhydrazine (FCCP; 10 μM , $n = 3$, 102 cells). By contrast, Ψ_{mito} was increased in *dLetm1* knockdown cells ($n = 3$, 113 cells) as compared to cells treated with scrambled control dsRNA ($n = 3$, 168 cells). **(D)** Relative mRNA level of *dLetm1* and actin in control and *dLetm1* dsRNA-treated S2 cells by quantitative reverse transcription-polymerase chain reaction (RT-PCR) ($n = 3$). **(E)** Ca^{2+} - and pH gradient-driven $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$ changes in permeabilized S2-pericam cells treated with control (circles; $n = 4$, 143 cells) or *dLetm1* dsRNAs (triangles; $n = 4$, 113 cells). A representative trace shows the effect of applying the $\text{H}^{+}/\text{K}^{+}$ antiporter nigericin (1 μM , dashed colored line) on *dLetm1* dsRNA-treated cells ($n = 3$, 87 cells). **(F)** pH-dependent $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$ changes in permeabilized S2-pericam cells treated with scrambled control (circles; $n = 6$, 214 cells) or *dLetm1* dsRNAs (triangles; $n = 6$, 187 cells). BAPTA-maintained test solution $[\text{Ca}^{2+}] = 50$ nM. A representative trace shows the effect of nigericin (1 μM , dotted line) on *dLetm1* dsRNA-treated cells ($n = 3$, 104 cells). All data shown are the mean $\pm$ SEM (* $P < 0.05$, two-tailed Student's *t* test).

responses. Mammalian uncoupling proteins (UCPs) were recently proposed to be critical for $\text{Ca}^{2+}_{\text{mito}}$ uptake (12) [but see (13)]. However, dsRNAs against *Drosophila* mitochondrial UCPs did not affect $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$ changes in our screen and subsequent assays (fig. S4).

Extramitochondrial $[\text{Ca}^{2+}]$ was clamped in permeabilized S2 cells in Na^{+} -free intracellular solution to abrogate potential mitochondrial Na^{+}

(H^{+} or $/\text{Ca}^{2+}$) exchangers. Basal $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$ were similar in control and dLetm1 knockdown cells under this condition (fig. S5). dLetm1 knockdown markedly reduced $\text{Ca}^{2+}_{\text{mito}}$ uptake at $<1 \mu\text{M}$ $[\text{Ca}^{2+}]_{\text{cyto}}$, a transport mode coupled with mitochondrial H^{+} extrusion (Fig. 1A). Free $[\text{Ca}^{2+}]_{\text{mito}}$ at steady state was $\sim 5 \mu\text{M}$, far below the calculated free $[\text{Ca}^{2+}]_{\text{mito}}$ equilibrium ($\sim 1 \text{ M}$, assuming $1 \mu\text{M}$ cytoplasmic $[\text{Ca}^{2+}]$),

indicating an intrinsic limit for this mode of uptake. At $[\text{Ca}^{2+}]_{\text{cyto}} > 1 \mu\text{M}$, a dLetm1-independent $\text{Ca}^{2+}_{\text{mito}}$ uptake mode became apparent (Fig. 1B). This rapid $\text{Ca}^{2+}_{\text{mito}}$ uptake mode stimulated by higher $[\text{Ca}^{2+}]_{\text{cyto}}$ was not associated with $\text{H}^{+}_{\text{mito}}$ extrusion and caused membrane depolarization, consistent with MCU/MiCa. dLetm1 knockdown had little effect on $100 \mu\text{M}$ $[\text{Ca}^{2+}]_{\text{cyto}}$ -induced $\text{Ca}^{2+}_{\text{mito}}$ influx (Fig. 1A).

$\text{Ca}^{2+}_{\text{mito}}$ uptake in cells lacking dLetm1 was not coupled with $\text{H}^{+}_{\text{mito}}$ extrusion. We measured voltage across the inner mitochondrial membrane (Ψ_{mito}) using tetramethyl rhodamine methyl ester (TMRM; 5 nM) to determine whether dLetm1 knockdown affected electron transport chain (ETC)-dependent proton export. Unlike ETC inhibitors that reduce Ψ_{mito} in control cells, resting Ψ_{mito} was increased in dLetm1-knockdown cells (Fig. 1, C and D). To summarize the results thus far, dLetm1 is crucial to $\text{Ca}^{2+}_{\text{mito}}$ uptake in low $[\text{Ca}^{2+}]$, and the pH gradient appears to intrinsically limit dLetm1-associated mitochondrial Ca^{2+} uptake.

To test the apparent limitation by the pH gradient, we studied pH-dependent changes on $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$. $\text{Ca}^{2+}_{\text{mito}}$ extrusion and $\text{H}^{+}_{\text{mito}}$ influx were induced by a decline in $[\text{Ca}^{2+}]_{\text{cyto}}$, and by cytoplasmic acidification (Fig. 1E). dLetm1 knockdown specifically abolished Ca^{2+} -dependent pH changes and pH-driven $\text{Ca}^{2+}_{\text{mito}}$ extrusion. As expected, alkalization induced an initial phase of rapid $\text{Ca}^{2+}_{\text{mito}}$ uptake and $\text{H}^{+}_{\text{mito}}$ extrusion, and a much slower second phase of $\text{Ca}^{2+}_{\text{mito}}$ uptake and $\text{H}^{+}_{\text{mito}}$ extrusion (Fig. 1F). The pH-dependent $\text{Ca}^{2+}_{\text{mito}}$ uptake was slowed by more than fourfold in dLetm1 knockdown cells. In the yeast *mdm38* (homolog of *Letm1* and *CG4589*) mutant, mitochondria were swollen and the phenotype could be rescued

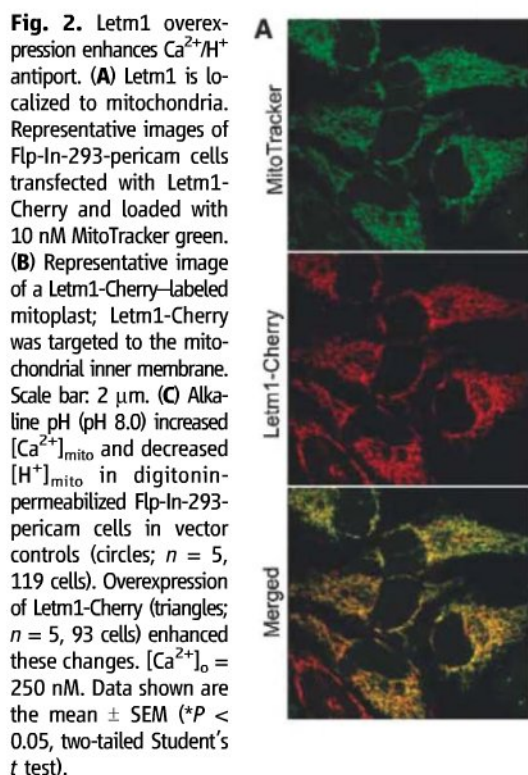
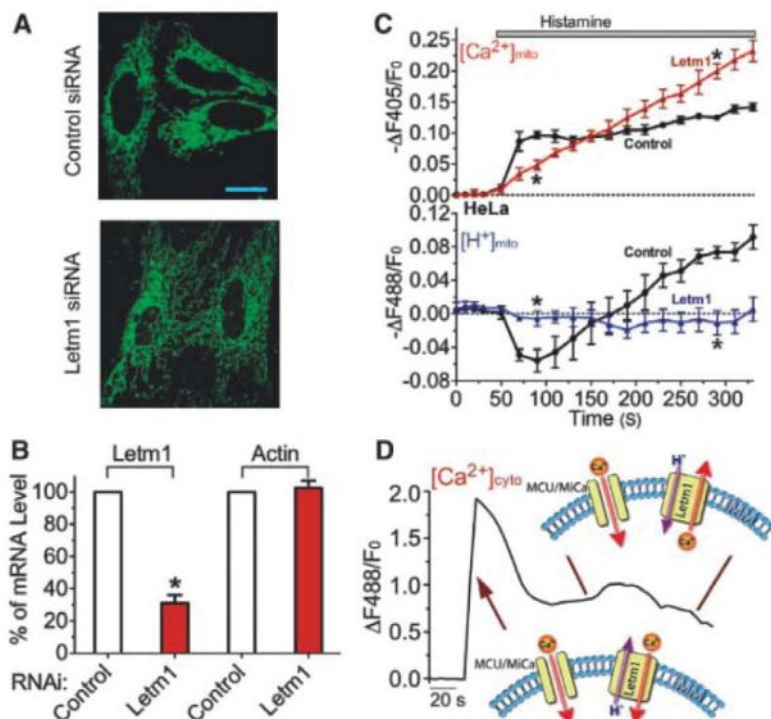


Fig. 3. Letm1 knockdown disrupts $\text{Ca}^{2+}/\text{H}^{+}$ antiport in intact cells. (A) mt-Pericam-labeled mitochondria appear grossly normal in a representative image of *Letm1* siRNA-treated HeLa cells compared to cells treated with scrambled control siRNA. Scale bar: $20 \mu\text{m}$. (B) Determination of the mRNA level of *Letm1* and actin in control and *Letm1* siRNA-treated HeLa cells by quantitative RT-PCR ($n = 3$). (C) Letm1 knockdown disturbs normal mitochondrial $[\text{Ca}^{2+}]_{\text{mito}}$ and $[\text{H}^{+}]_{\text{mito}}$ regulation in H1R-expressing HeLa cells. Histamine was applied to stimulate an increase in cytoplasmic $[\text{Ca}^{2+}]$ in cells in $[\text{Ca}^{2+}]_o = 2 \text{ mM}$ treated with control (circles; $n = 8$, 76 cells) or *Letm1* siRNAs (triangles; $n = 6$, 47 cells). Two independent *Letm1* siRNAs were used to confirm the result. (D) Representative trace of the $[\text{Ca}^{2+}]_{\text{cyto}}$ changes in HeLa cells upon histamine stimulation, and model of the roles of MCU/MiCa and Letm1 $\text{Ca}^{2+}/\text{H}^{+}$ exchanger activity under these conditions (arrows indicate their actions during the trace). All data shown are the mean $\pm$ SEM ($*P < 0.05$, two-tailed Student's t test).



by the exogenous K^+/H^+ exchanger, nigericin (14). In these studies, however, active mitochondrial K^+/H^+ exchange was only observed under low-divalent or divalent-free conditions (14). In our experiments in dLetm1 knockdown S2 cells, application of nigericin augmented H^+ flux (Fig. 1, E and F), but did not rescue the loss of pH-driven Ca^{2+} exchange.

The human homolog of CG4589, *Letm1* (leucine zipper EF-hand-containing transmembrane protein 1), is an evolutionarily conserved, ubiquitously expressed, homomeric inner mitochondrial membrane protein of unclear function (15–18). In humans, partial deletion of the short arm of chromosome 4 (including *Letm1*) results in Wolf-Hirschhorn syndrome (WHS), characterized by mental retardation, microcephaly, seizures, hypotonia, and cleft lip/palate (19). In mammalian cells, Cherry-tagged Letm1 was exclusively localized to the mitochondrial inner membrane (Fig. 2, A and B). In cells overexpressing Letm1, pH-driven Ca^{2+} uptake and H^+ extrusion were accelerated by more than fivefold (Fig. 2C). Mitochondrial H^+ transport was not secondary to $[Ca^{2+}]_{mito}$ alone, but rather to the Ca^{2+} gradient across the inner mitochondrial membrane (fig. S6).

Most HeLa cells with short-term (~3 days) *Letm1* small interfering RNA (siRNA) treatment appeared healthy and their mitochondria appeared normal under light microscopy (Fig. 3A), despite an ~70% reduction in mRNA level (Fig. 3B). Knockdown of Letm1 abolished bidirectional mitochondrial Ca^{2+}/H^+ antiport, resulting in reduced initial Ca^{2+} uptake and late Ca^{2+} overload (Fig. 3, C and D), supporting the essential role of Letm1 in Ca^{2+}/H^+ antiporter function and $[Ca^{2+}]_{mito}$ homeostasis. As in S2 cells, knockdown of Letm1 did not cause a decrease, but rather a slight increase, in resting Ψ_{mito} (fig. S7), consistent with a previous report that Letm1 knockdown does not impair ETC function (17).

To directly test whether the Letm1 protein mediates Ca^{2+}/H^+ antiport, C-terminal His-tagged Letm1 was expressed in bacteria, purified (Fig. 4A), and incorporated into liposomes. Letm1-containing liposomes rapidly accumulated Ca^{2+} . Ca^{2+} uptake was blocked by Ruthenium red (and Ru360), nonselective inhibitors of divalent cation channels and transporters (Fig. 4B). CGP-37157 (~4 μ M), a nonselective inhibitor of Na^+/Ca^{2+} exchangers, inhibited the transport rate by ~25%. Addition of Ca^{2+} induced H^+ efflux; also transiently increasing external pH induced rapid H^+ efflux from Letm1-liposomes (Fig. 4C). In symmetrical $[Ca^{2+}]$, external acidic pH drove Ca^{2+} release (Fig. 4D), whereas alkaline pH induced Ca^{2+} uptake (Fig. 4E). The maximum rate of Ca^{2+} transport (V_{max}) by isolated Letm1 protein was 4.1 nmol/ μ g protein per second [~1700 ions per second, assuming that Letm1 is tetrameric (22, 23)], reaching half-saturation at 132.7 nmol Ca^{2+} / μ g protein; Michaelis constant (K_m) = 137 nmol Ca^{2+} / μ g protein (Fig. 4F). Ca^{2+} transport velocity was increased about sevenfold

by increasing the transliposomal potential (fig. S8), suggesting that Letm1 is electrogenic. These results, using isolated Letm1-containing liposomes, strongly suggest that Letm1 itself comprises a Ca^{2+}/H^+ antiporter.

We showed that Letm1 is a Ca^{2+}/H^+ exchanger in S2 cells, mammalian cells, and as an isolated protein in proteoliposomes. Letm1 exchanges Ca^{2+} for H^+ at submicromolar $[Ca^{2+}]_{cyto}$. When $[Ca^{2+}]_{mito}$ is high, such as after MCU/MiCa activation, or when cytoplasmic pH is low, Letm1 should extrude excess Ca^{2+} and acidify the mitochondrial matrix. When $[Ca^{2+}]_{mito}$ is low, Ca^{2+} will be imported and the mitochondrial matrix alkalinized. Letm1 Ca^{2+}/H^+ exchange was not sensitive to $[Na^+]$. Prior evidence for Na^+ -dependent

Ca^{2+} extrusion suggests that a third molecule, probably a Na^+/Ca^{2+} exchanger, is also present in the inner mitochondrial membrane (1, 4, 5). We speculate that the relative amounts of these three Ca^{2+} -exchange mechanisms vary in different cell types, correlating with their cell-specific functions.

We found that long-term Letm1 knockdown with repetitive siRNA application resulted in morphological changes in certain HeLa cell subpopulations (~20% cells). However, other studies showed no mitochondrial morphology changes in lymphoblastoid cells and primary fibroblasts from WHS patients despite a notable reduction in the amount of Letm1 protein (17). These data suggest that mitochondrial morphology changes

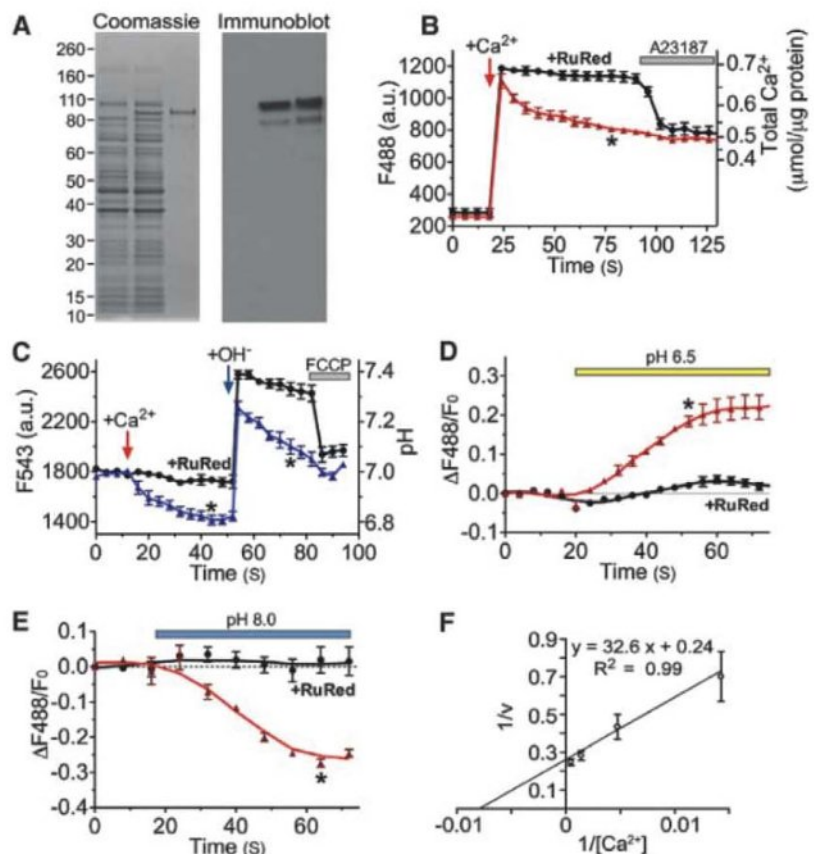


Fig. 4. Purified Letm1 reconstitutes Ca^{2+} transport in liposomes. (A) Letm1 protein stained by Coomassie blue (left) and antibody to His-Letm1 (right). Left lane, total bacterial cell lysate from control; middle lanes, Letm1-His-expressing bacteria; right lanes, isolated Letm1-His proteins. The band migrating at ~83 kD is consistent with Letm1's predicted molecular size. (B) Ca^{2+} addition initially increased external Ca^{2+} , followed by Ca^{2+} import as indicated by decreasing fluorescence (triangles; $n = 8$). Ca^{2+} uptake was blocked by Ruthenium red (RuR; 10 nmol/ μ g; circles; $n = 5$), which was reversed by the Ca^{2+} ionophore, A23187 (5 μ M). Liposomes occupied ~30% of the total volume based on the maximum Ca^{2+} uptake triggered by 4-Bromo-A23187. No leak was detected in liposomes without Letm1. (C) Ca^{2+} -driven H^+ efflux in Letm1 proteoliposomes. Addition of 100 μ M Ca^{2+} triggered H^+ efflux; application of 100 μ M CsOH transiently increased the external pH, followed by a rapid decline in pH (triangles; $n = 5$), which was blocked by RuR (10 nmol/ μ g; circles; $n = 3$). Finally, FCCP (10 μ M, protonophore) reduced the external pH to basal levels. (D and E) pH-driven Ca^{2+} uptake in Letm1 proteoliposomes; Ca^{2+} release or uptake in Letm1 proteoliposomes was blocked by RuR. No-added-RuR, triangles, $n = 4$; 10 nmol/ μ g RuR, circles, $n = 3$. (F) The inverse Ca^{2+} transport velocity ($1/v$; nmol/ μ g protein per second) was plotted against inverse total added $[Ca^{2+}]$ (nmol/ μ g protein, $n = 3$ to 8) in this double reciprocal plot to calculate K_m (137 nmol Ca^{2+} / μ g protein) and V_{max} (4.2 nmol/ μ g protein per second) using Michaelis-Menten assumptions (24). All data shown are the mean $\pm$ SEM (* $P < 0.05$ in a two-tailed Student's t test).

are secondary to the loss of Letm1, but may depend on cell type.

Letm1 has no appreciable homology to the bacterial and plant CaX family of $\text{Ca}^{2+}/\text{H}^{+}$ or $\text{Ca}^{2+}/\text{Na}^{+}$ transporters. Based on our preliminary analysis of the potential topologies using *Robetta* (fig. S9) (20), we hypothesize that Letm1 resembles the inner mitochondrial membrane Mg^{2+} transporter (21), whose bacterial homolog (CorA) forms a funnel-shaped structure (22, 23).

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- We thank B. Mathey-Prevot, N. Ramadan, M. Booker, and staff at the Drosophila RNAi Screening Center for assistance with the screen; A. Miyawaki for pericam; R. Tsien for CherryFP; T. Schwarz for the PMK33 vector; R. Kaplan for advice on the proteoliposome study; D. Baker for assistance with *Robetta*; and G. Kravitsky, Y. Kirichok, and J. Jin for discussions. D.J. is a recipient of fellowship awards from the Canadian Institutes of Health Research and the Alberta Heritage Foundation for Medical Research.

Supporting Online Material

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SOM Text

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20 April 2009; accepted 4 August 2009

10.1126/science.1175145

Dissecting the Genetic Basis of Resistance to Malaria Parasites in *Anopheles gambiae*

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The ability of *Anopheles gambiae* mosquitoes to transmit *Plasmodium* parasites is highly variable between individuals. However, the genetic basis of this variability has remained unknown. We combined genome-wide mapping and reciprocal allele-specific RNA interference (rasRNAi) to identify the genomic locus that confers resistance to malaria parasites and demonstrated that polymorphisms in a single gene encoding the antiparasitic thioester-containing protein 1 (TEP1) explain a substantial part of the variability in parasite killing. The link between TEP1 alleles and resistance to malaria may offer new tools for controlling malaria transmission. The successful application of rasRNAi in *Anopheles* suggests that it could also be applied to other organisms where RNAi is feasible to dissect complex phenotypes to the level of individual quantitative trait alleles.

Anopheles gambiae mosquitoes are major vectors of *Plasmodium falciparum*, a protozoan parasite that causes the most severe form of human malaria in Africa. The fact that mosquito strains that are completely resistant to malaria parasites can be selected (1, 2) indicates that genetic factors in mosquitoes control the level of parasite transmission. Understanding the genetic basis of this resist-

ance has been a long-standing question. The L3-5 resistant strain kills and melanizes a wide variety of parasite species (1). Previous genetic analyses of crosses between this strain and the susceptible 4Arr strain infected with two simian parasite species focused on the melanotic encapsulation phenotype and identified several quantitative trait loci (QTLs), whose relative contributions varied with parasite species and between F2 generation families (3, 4). Recently, it became clear that melanization occurs after parasite killing as a means to dispose of dead parasites in some strains, whereas in others, killed parasites are only cleared by lysis (fig. S2A) (5–7). In this study, we aimed at mapping the genomic regions and identifying genes that control resistance (the absence of live parasites) of mosquitoes to the rodent malaria parasite *Plasmodium berghei*.

We set up reciprocal crosses of the resistant L3-5 and susceptible 4Arr strains. F1 mosquitoes were intercrossed, and individual females were

isolated to lay eggs, yielding 10 F2 families. Females were blood-fed on mice infected with *PbGFPcon*, a transgenic clone of *P. berghei* expressing GFP constitutively (8). Fluorescent live and dead melanized parasites were counted on dissected midguts 7 to 9 days post infection [Fig. 1A and supporting online material (SOM) text]. As expected, parental L3-5 females displayed only melanized parasites (with the exception of one that bore one live parasite), and 4Arr mosquitoes displayed only live parasites. Most of the 111 F1 mosquitoes exhibited an intermediate phenotype (mix of live and melanized parasites). Both parental and F1 phenotypes were present in the 402 F2 females. Percentages of resistant (devoid of live parasite) and melanizing (bearing at least one melanized parasite) mosquitoes in each generation (Fig. 1B) did not follow the segregation pattern of simple Mendelian traits [$P < 0.001$ in both cases (9)], indicating that the killing of *P. berghei* and the mode of clearance of dead parasites are complex traits that are each likely to result from the segregation of several alleles.

To map loci controlling resistance to parasites, we genotyped 39 informative markers spanning the entire genome in 206 selected F2 individuals with extreme phenotypes (fig. S1 and SOM text). Linkage analysis comparing resistant and non-resistant mosquitoes identified a single region on chromosome 3L (Fig. 1C). We interpreted this region, covering ~19 Mb, as a major locus responsible for resistance to *P. berghei* and named it *Pbres1* for *P. berghei* resistance locus 1. We further compared the genotypes of melanizing and nonmelanizing mosquitoes and detected two intervals that are likely to contain regulators affecting the mode of clearance of dead parasites (i.e., the balance between lysis and melanization) (Fig. 1C): (i) a major QTL on chromosome 2R, which we named *Pbmell* (5 Mb) for *P. berghei* melanization locus 1, and (ii) a minor pericentromeric QTL on chromosome 3, *Pbmell2* (17 Mb), that partially overlaps with *Pbres1*. Linkage map-

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ping using the actual counts of live or melanized parasites identified the same loci as above (fig. S2B and SOM text). The newly identified QTLs overlap with regions previously identified as controlling melanization of *P. cynomolgi* and *P. berghei* in L3-5 mosquitoes (Fig. 1C) (3, 4, 10), indicating that the major mechanisms underlying parasite elimination in L3-5 are likely partially conserved and independent of parasite species. Nevertheless, clear quantitative differences exist between the four studies, probably, at least in part, because previous studies did not consider resistance and melanization as distinct traits.

Because of its major role in parasite transmission, we investigated the resistance QTL on chromosome 3L in more detail. *Pbres1* contains ~975 genes, among which 35 can be classified as “immune-related” (11). This category includes the gene encoding the thioester-containing protein 1 (TEP1), a complement-like molecule circulating in the hemolymph with key antiparasitic activity (12). Two features make it an attractive candidate: (i) it binds to and promotes the killing of midgut stages of the rodent parasite *P. berghei*, and (ii) it is highly polymorphic (5). To examine

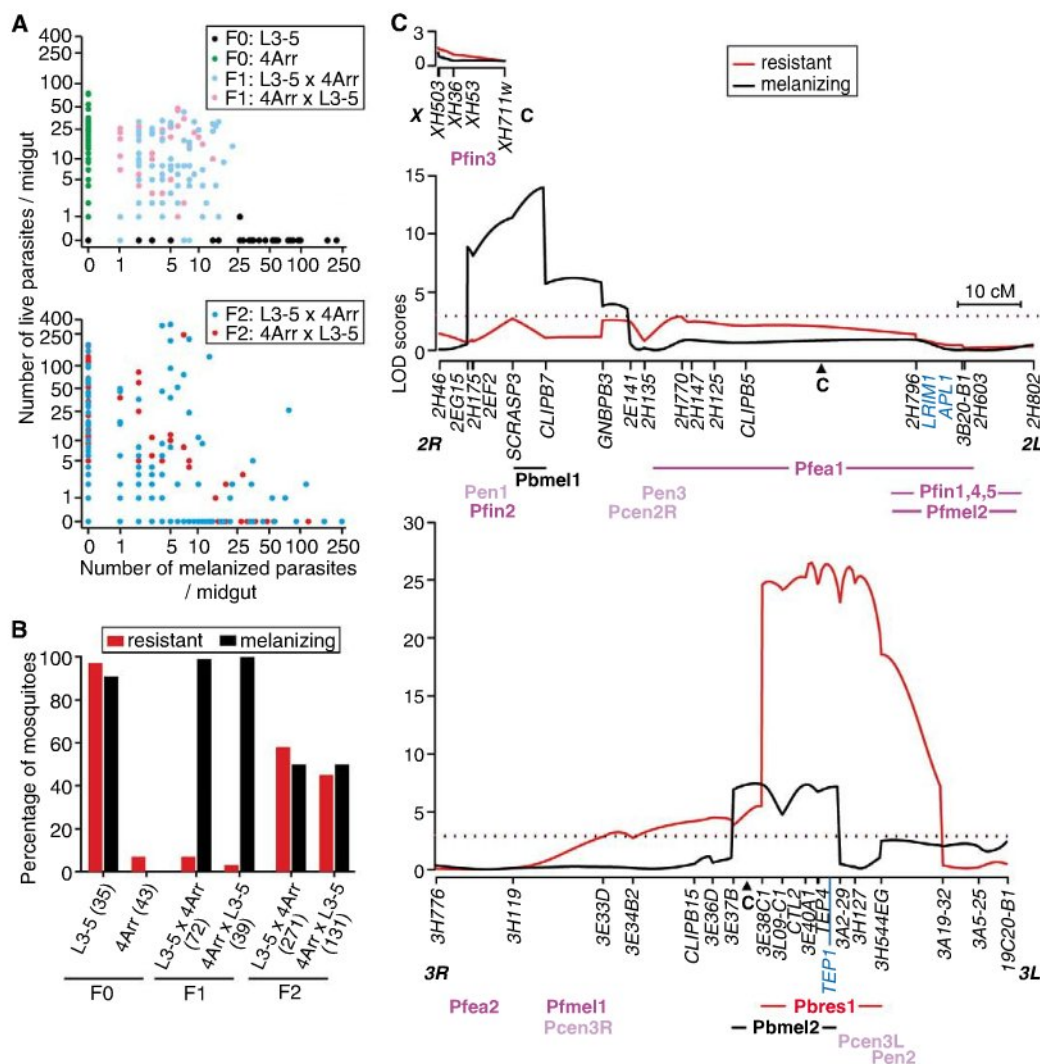
TEP1 polymorphism in the L3-5 and 4Arr strains, we cloned and sequenced the full open reading frame (Fig. 2 and fig. S3). We renamed the previously known *TEP1r* (or *TEP16*) from the L3-5 strain (5, 13) as *TEP1*R1*, and we renamed *TEP1s* (or *TEP1*) from the PEST strain (14) as *TEP1*S1*. All *TEP1* sequences in L3-5 mosquitoes were identical to **R1*. Sequences from the 4Arr strain appeared to be chimeras of *TEP1*S1* and *TEP1*R1*: One was closer to **S1*, thus we named it *TEP1*S2*; the second allele clustered with **R1* in the phylogenetic tree and was therefore named *TEP1*R2*. We also sequenced *TEP1* from our G3 colony and confirmed that it was closely related, although not identical, to *TEP1*S1*. We named this allele *TEP1*S3*.

To determine whether the diverse alleles of *TEP1* have a phenotypic effect, we compared the degree of resistance of mosquitoes that differed solely in the expression of *TEP1* alleles. For this, we developed an assay similar to reciprocal hemizygosity analysis in yeast (15): We used reciprocal allele-specific RNA interference (rasRNAi) instead of chromosomal deletions to silence each allele separately in F1 mosquitoes,

enabling us to compare the function of each allele in the same genetic background (Fig. 3A). We crossed resistant L3-5 and susceptible G3 mosquitoes, as these strains are homozygous for *TEP1* and bear representative alleles of the *TEP1*R* and *TEP1*S* classes. Additionally, G3 and 4Arr mosquitoes share the same susceptible phenotype.

We designed three pairs (a to c) of short double-stranded RNAs (dsRNAs; *dsR/dsS*) specifically targeting **R1* and **S3* and tested them in the parental L3-5 and G3 strains (fig. S4 and SOM text). We used *dsLacZ* as a negative control and *dsTEP1* that targets both alleles as a positive control (5). Pair a (*dsRa* and *dsSa*) was selected for further experiments: 3 to 4 days after dsRNA treatment, *TEP1*R1* was depleted from L3-5 mosquitoes upon treatment with *dsRa*, but not *dsSa*, and reciprocally, injection of *dsSa*, but not *dsRa*, in G3 mosquitoes reduced *TEP1*S3* levels. In the F1 progeny of reciprocal crosses between L3-5 and G3 mosquitoes, both alleles were silenced to a similar level by allele-specific RNAi, allowing us to specifically study the function of each allele (Fig. 3B, fig. S4B, and SOM text).

Fig. 1. Loci associated with resistance and clearance of dead parasites. (A) Numbers of melanized (x axis) and live (y axis) parasites per mosquito in reciprocal crosses of the resistant L3-5 and susceptible 4Arr strains. (B) Percentages of resistant (devoid of live parasites) and melanizing (bearing at least one melanized parasite) mosquitoes in each generation. (C) Linkage mapping for the resistant (red) and melanizing (black) traits, with estimated logarithm of the odds ratio for linkage (LOD score) thresholds represented as dotted lines (3.00 and 2.88, respectively). Genetic markers; centromere positions (C); chromosome arms; and the *TEP1*, *LRM1*, and *APL1* loci (light blue) are indicated below the axes. Previously identified QTLs for resistance against simian parasites (light purple) or *P. falciparum* (dark purple) are positioned below the chromosomes. The PRI corresponds to the region covered by the QTLs Pfin1, Pfin4, Pfin5, and Pfmel2.



dsRNA-treated F1 mosquitoes were infected on mice carrying *PbGFPcon* (Fig. 3C and fig. S4C). Control *dsLacZ*-treated F1 mosquitoes bore a mixture of live and melanized parasites. **R1*-depleted mosquitoes (*dsRa*) were significantly more susceptible than **S3*-depleted (*dsSa*) mosquitoes and were completely unable to melanize. Moreover, **S3*-depleted mosquitoes were consistently more resistant than *dsLacZ* controls, containing fewer live parasites. Thus, *TEP1***R1* is more efficient than *TEP1***S3* in promoting parasite killing and melanization of dead parasites. The reversal of the F1 phenotype toward the susceptible parent phenotype upon depletion of *TEP1***R1*, or toward the resistant parent phenotype upon depletion of *TEP1***S3*, indicates that polymorphisms in *TEP1* are a major determinant of resistance to *P. berghei* in these mosquito strains.

To examine whether the two allelic variants of the 4Arr strain (**S2* and **R2*, which belong to the *TEP1***S* and *TEP1***R* classes) also differ in their efficiency in parasite killing, we further refined our association analysis of the F2 progeny of the QTL mapping crosses and genotyped all F2 progeny for *TEP1* (Fig. 4 and SOM text). Most **R1/R1* F2 mosquitoes (81%) were fully resistant, and those that were not carried only a few live parasites. In contrast, 90% of **S2/S2* mosquitoes were susceptible, containing high parasite loads. **R2/R2* mosquitoes had an intermediate phenotype, suggesting that although **R2*

is closely related to **R1*, the few polymorphisms between these two alleles affect its efficiency in parasite killing. Further studies are needed to precisely identify the essential single-nucleotide polymorphism(s) and the molecular mechanisms that underlie this resistance. In addition, **R1/R2* mos-

quitoes were more resistant than **R1/S2* mosquitoes, indicating that the two 4Arr alleles confer different degrees of resistance, with **R2* > **S2*. Thus, the complexity of the resistance inheritance in our crosses is partially explained by the segregation of the three *TEP1* alleles. Still, other

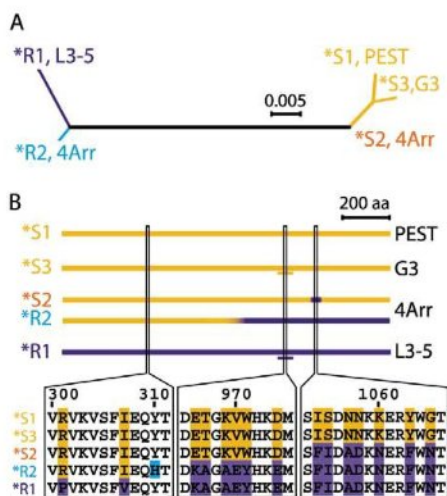


Fig. 2. *TEP1* polymorphism. (A) Phylogenetic tree built from the global alignment of complete amino acid sequences of *TEP1* alleles from L3-5 (**R1*), 4Arr (**R2* and **S2*), and G3 (**S3*) mosquitoes and the previously described **S1* allele from the PEST strain. Scale bar indicates estimated amino acid substitutions per site. (B) Schematic representation of *TEP1* sequences. Amino acid sequences of **S1* and **S3* are represented by orange horizontal bars, **R1* by a blue bar. The 4Arr alleles are combinations of **S1/S3* and **R1*, as illustrated by short stretches of aligned sequences. The short horizontal lines below **R1* and **S3* indicate the regions targeted by *dsRa* and *dsSa*, respectively.

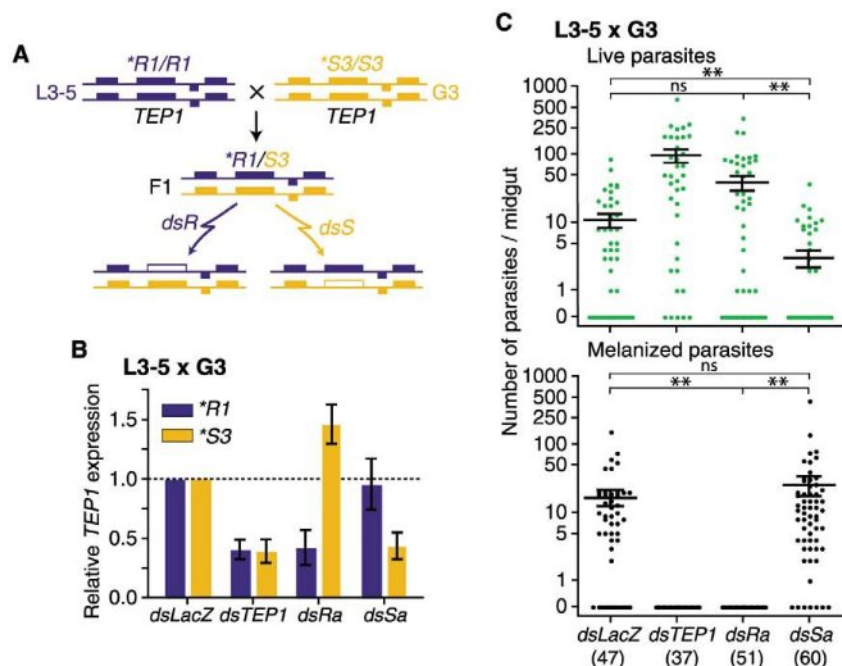


Fig. 3. *TEP1***R1* is more efficient than *TEP1***S3* in parasite killing. (A) *rasRNAi*. Each box represents a gene. With the use of short dsRNA probes specifically directed against **R1* (*dsR*) or **S3* (*dsS*), each *TEP1* allele is silenced separately in F1 mosquitoes (open box), allowing us to compare the function of each allele in the same genetic background. (B) *TEP1* expression in the F1 progeny of crosses between L3-5 females and G3 males (L3-5 × G3) was measured by allele-specific quantitative real-time polymerase chain reaction 3 days after dsRNA treatment. Expression levels of *TEP1***R1* and **S3* were normalized to their levels in the *dsLacZ* control. Mean (central bar) ± SEM (error bar) of three independent experiments. (C) Parasite counts in the F1 progeny of L3-5 × G3. The results of three independent experiments were pooled, and sample sizes are shown in brackets. Mean (central bar) ± SEM (error bar). Significance for differences between groups are indicated (Mann-Whitney on key comparisons): ***P* < 0.001; ns, not significant.

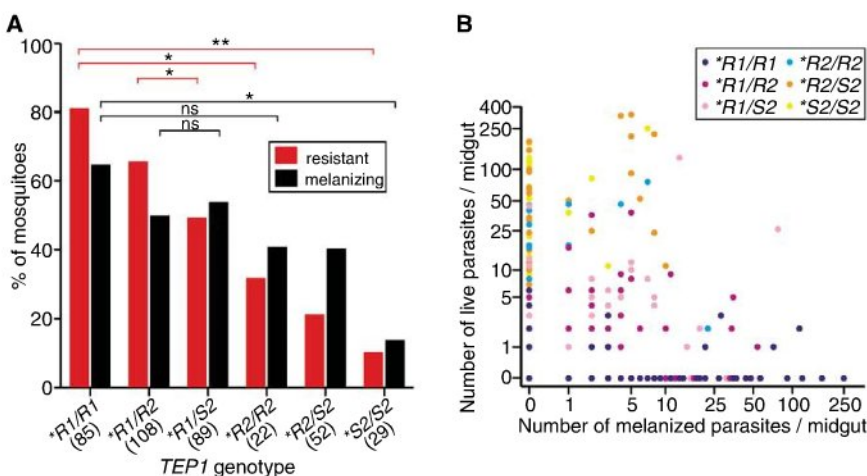


Fig. 4. Correlation between *TEP1* genotype and phenotype upon *P. berghei* infection in the F2 generation. (A) Percentages of resistant and melanizing mosquitoes for each genotype. Sample sizes are shown in brackets. Significance for differences between groups were calculated taking into account F2-family structure (9): ***P* < 0.001; **P* < 0.05; ns, not significant. (B) Parasite counts in F2 mosquitoes as shown in Fig. 1A.

genes besides *TEP1* must contribute. This is apparent from comparing phenotypes of groups from different generations with the same *TEP1* genotypes (Figs. 1B and 4A): For instance, 50 to 70% of **R1/R2* and **R1/S2* mosquitoes were resistant in F2, whereas <7% were resistant in F1. Thus, this additional locus (or loci) appears to be unlinked to *TEP1* and also to have a limited impact in mosquitoes homozygous for the extreme alleles **R1* and **S2*, which have similar resistance as the parental strains but are essential to support resistance in heterozygotes. Future work may focus on identifying secondary QTL(s) and potential candidate *TEP1* suppressor gene(s).

The single locus identified here that controls resistance to *P. berghei* and includes *TEP1* does not overlap with previously reported QTLs controlling the intensity of infection of natural populations by the human malaria parasite *P. falciparum*, and in particular, does not overlap with the major *Plasmodium* resistance island (PRI) (16–18) (Fig. 1C). Two leucine-rich repeat proteins encoded in the PRI, APL1 and LRIM1, form a complex with *TEP1*. These proteins maintain mature *TEP1* in circulation and regulate its binding to parasites and their subsequent killing (19, 20). Polymorphisms in *TEP1* itself or in proteins that control *TEP1* function might both contribute to the efficiency of *TEP1* antiparasitic activity. The differences between the QTLs identified in laboratory strains and in field mosquitoes might thus reflect the sampling of determinant polymorphism(s) in various players of the same pathway, rather than different mechanisms em-

ployed to limit development of human and rodent malaria parasite species. Consistently, silencing of *TEP1* increases *A. gambiae* susceptibility to both murine and human *Plasmodia* (5, 21). Haplotypes of the susceptible and resistant alleles of *TEP1*, as well as recombinants between these forms, exist in field populations from East and West Africa (22). Understanding the genetic basis of resistance to malaria parasites, as well as how the determinant polymorphisms are maintained and selected in field populations, will be of tremendous importance for the control of malaria transmission.

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23. We thank A. Cohuet for sharing sequencing information, R. Carmouche and J. Luis from the EMBL Genecore facility for support with genotyping, R. Bourgon for advice on statistical analyses, A. Budd for suggestions on the phylogenetic analysis, D. Doherty and J. Soichot for help with breeding mosquitoes, and E. Marois and C. Ramakrishnan for comments on the manuscript. We acknowledge the support of F. Kafatos, in whose laboratory this work was initially carried out. This work was supported by grants from NIH and the Deutsche Forschungsgemeinschaft (L.M.S.), CNRS and INSERM (E.A.L. and S.A.B.), the European Molecular Biology Organization (EMBO) Young Investigators Program (E.A.L.), and the European Commission Network of Excellence "BioMalPar" (E.A.L.). S.A.B. received a postdoctoral fellowship from EMBO. E.A.L. is an international Howard Hughes Medical Institute research scholar. EMBL Nucleotide Sequence Database accession numbers for *TEP1* alleles sequenced in this report are as follows: FN431782 to FN431785.

Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5949/147/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 to S3
References

21 April 2009; accepted 5 August 2009
10.1126/science.1175241

Coat Variation in the Domestic Dog Is Governed by Variants in Three Genes

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Coat color and type are essential characteristics of domestic dog breeds. Although the genetic basis of coat color has been well characterized, relatively little is known about the genes influencing coat growth pattern, length, and curl. We performed genome-wide association studies of more than 1000 dogs from 80 domestic breeds to identify genes associated with canine fur phenotypes. Taking advantage of both inter- and intrabreed variability, we identified distinct mutations in three genes, *RSPO2*, *FGF5*, and *KRT71* (encoding R-spondin-2, fibroblast growth factor-5, and keratin-71, respectively), that together account for most coat phenotypes in purebred dogs in the United States. Thus, an array of varied and seemingly complex phenotypes can be reduced to the combinatorial effects of only a few genes.

The tremendous phenotypic diversity of modern dog breeds represents the end point of a >15,000-year experiment in artificial and natural selection (1, 2). As has been demonstrated for traits such as body size (3) and

coat color (4), marker-based associations with phenotypic traits can be explored within single breeds to initially identify regions of genetic association, and then expanded to multiple breeds for fine-mapping and mutation scanning (5, 6).

Coat (pelage) phenotypes are particularly amenable to this strategy as they show a huge amount of variation across breeds but still allow for simple variation within single breeds (7). This offers a unique strategy for advancing the genetic understanding of a complex phenotype.

We used the structured pattern of fur variation in dogs to localize the genetic basis of three characteristics of the canine coat: (i) the presence or absence of "furnishings," the growth pattern marked by a moustache and eyebrows typically observed in wire-haired dogs; (ii) hair length; and (iii) the presence or absence of curl. To accomplish this, we generated three genome-wide single-

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nucleotide polymorphism (SNP) data sets using the Affymetrix version 2.0 canine SNP chip (8, 9). The first data set consisted of 96 dachshunds segregating three coat varieties: wire-haired with furnishings, smooth, and long-haired without furnishings. The second data set comprised 76 Portuguese water dogs (PWDs), segregating the curl phenotype. The final data set, termed CanMap, included 903 dogs from 80 breeds representing a wide variety of phenotypes. An additional data set used to map furnishings included a panel of microsatellite markers (10), genotyped on a 96-dachshund pedigree segregating all three coat varieties.

The same strategy was used to map all three traits. First, a genome-wide association study (GWAS) within a breed segregating the phenotype was conducted to determine the most strongly associated locus. To rule out false-positives caused by population structure within the breeds (11), we did a second GWAS that used the CanMap data set divided into cases and controls

based on the presence or absence of the phenotype in question. Fine-mapping of significant, concordant peaks was used to define the smallest shared haplotype, followed by sequencing to identify the putative causative mutations. Each mutation was validated in a large panel of at least 661 dogs from 108 breeds, including cases and controls for all phenotypes (table S1).

We initially mapped furnishings in the dachshund using smooth-coated and long-haired dogs as controls and wire-haired dogs as cases (Fig. 1A). Single-marker analysis of the dachshund GWAS data set and concurrent linkage analysis of the dachshund pedigree identified the same locus on canine chromosome 13 (CFA13) surrounding nucleotide 11,095,120 [$P = 3.4 \times 10^{-27}$, lod score (logarithm of the odds ratio for linkage) = 5.6; Fig. 1B]. We confirmed the association on CFA13 in the CanMap data set at nucleotide 11,659,792 ($P = 10^{-241}$; Fig. 1C and table S2). A 718-kb homozygous haplotype in all dogs fixed with furnishings was located within both

the original 3.4-Mb haplotype observed in the dachshund-only GWAS, and a 2.8-Mb haplotype identified in crossover analysis within the dachshund pedigree (Fig. 1D).

Fine-mapping allowed us to reduce the homozygous region to 238 kb spanning only the R-spondin-2 (*RSPO2*) gene, excluding the 5' untranslated region (5'UTR) and the first exon (Fig. 1D, fig. S1, and table S3). *RSPO2* is an excellent candidate for a hair-growth phenotype as it synergizes with *Wnt* to activate β -catenin (12), and *Wnt* signaling is required for the establishment of the hair follicles (13, 14). Moreover, the *Wnt*/ β -catenin pathway is involved in the development of hair-follicle tumors, or pilomatricomas (15), which occur most frequently in breeds that have furnishings (16). Recent studies have shown that a mutation in the *EDAR* gene, also involved in the *Wnt* pathway, is responsible for a coarse East-Asian hair type found in humans (17), with some similarity to canine wirehair.

All exons and conserved regions of *RSPO2* were sequenced in dogs from seven breeds (table S4). Only an insertion of 167 base pairs (bp) within the 3'UTR at position 11,634,766 was perfectly associated with the furnishings trait in dogs from both the case/control study and the extended pedigree (table S5). The result was further confirmed in a set of 704 dogs of varying phenotypes. In total, 297 of 298 dogs with furnishings were either homozygous (268) or heterozygous (29) for the insertion, and all 406 dogs lacking the trait were homozygous for the ancestral state, as is consistent with a dominant mode of inheritance (table S1).

This mutation does not affect the protein-coding region of the *RSPO2* gene. However, because the 3'UTR frequently encodes elements that influence mRNA stability [reviewed in (18)], we examined whether the insertion was associated with a change in the expression level of the *RSPO2* gene. We found a threefold increase in *RSPO2* transcripts in muzzle skin biopsies of dogs with furnishings, consistent with a transcript effect (fig. S2).

We applied the same mapping strategy to hair length. Previously, mutations in the *FGF5* gene were identified in Welsh corgis segregating an atypical "fluffy" or long-haired phenotype (19) and associated with excess hair growth in mice and cats (20–22). Our study replicates these findings in an extended breed set. Indeed, association analyses in both the dachshund and CanMap data sets highlight the region on CFA32 containing *FGF5* with P values of 3×10^{-27} and 9×10^{-44} , respectively. After fine-mapping, a 67-kb homozygous region highlighted the *FGF5* gene (Fig. 2A, fig. S3, and table S6). The strongest association was observed at position 7,473,337 ($P = 1 \times 10^{-157}$), in which a highly conserved Cys is changed to Phe (Cys⁹⁵→Phe) in exon 1 of *FGF5*, consistent with the previous study (19). Sequencing within the homozygous haplotype revealed no SNPs with stronger association (table S7).

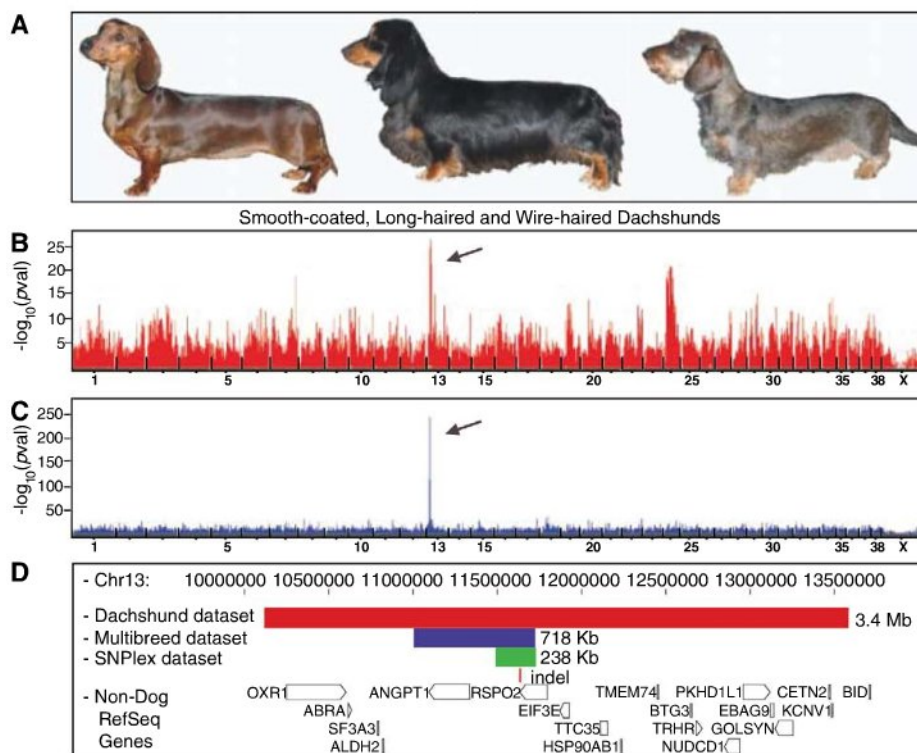


Fig. 1. GWAS and fine-mapping identify *RSPO2* as the associated gene for moustache and eyebrow growth pattern (furnishings). (A) Three types of coat segregate in dachshunds: (from left to right) smooth-coated, long-haired, and wire-haired with furnishings. (B) Results of the GWAS in the dachshund using wire-haired dogs as cases and smooth-coated and long-haired dogs as controls. The best P value (3.35×10^{-27}), highlighted by the arrow, is located on CFA13 at position 11,095,120. (C) Results of the GWAS for furnishings in the CanMap data set. The arrow highlights the best association ($P = 10^{-241}$) at CFA13 position 11,659,792. Both P values were obtained with single-marker χ^2 analyses. (D) Homozygous regions identified in cases from GWAS and fine-mapping. The red rectangle represents the associated haplotype in the dachshund; the blue rectangle spans the homozygous region from 19 breeds fixed for furnishings based on the multibreed data set; the green rectangle indicates the region of homozygosity in 18 breeds fixed for the furnishings after fine-mapping. A 167-bp deletion, indicated by the small red rectangle, is located within all three haplotypes in the 3'UTR of the *RSPO2* gene. The positions of genes in the region are represented by open boxes at the bottom of the figure, labeled to the left, with arrows indicating reading-frame direction (www.genome.ucsc.edu).

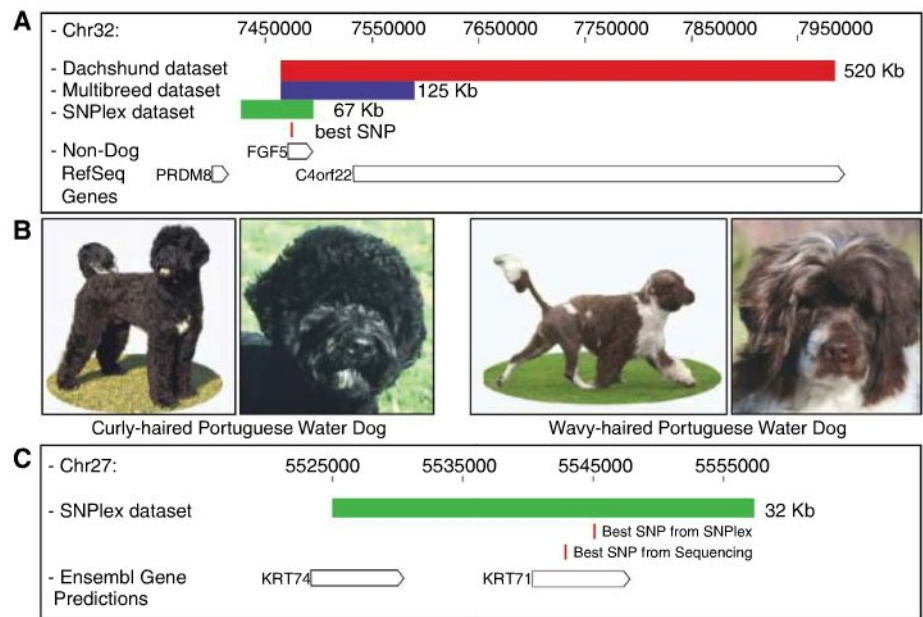


Fig. 2. Regions of homozygosity identify genes for pelage length and curl. **(A)** Homozygous region found on CFA32 defining the length locus. The red bar indicates the 520-kb associated haplotype from 29 long-haired dachshunds; the blue bar spans the 125-kb homozygous region found in 319 dogs from 31 long-haired breeds; the green bar represents the 67-kb reduced homozygous region found after fine-mapping in 293 dogs from 39 long-haired breeds. The best-associated SNP, represented by the small red rectangle, is within these three homozygous regions in exon 1 of the *FGF5* gene. **(B)** PWDs display two coat varieties: curly (two left panels) and wavy (two right panels). **(C)** Haplotype analysis at the curl locus on CFA27. The green bar represents the 32-kb homozygous haplotype found after fine-mapping in 65 dogs from five curly haired breeds. The best-associated SNP, represented by the small red rectangle, was found within the homozygous haplotype in exon 2 of the *KRT71* gene. The positions of genes in both regions (A and C) are represented by open boxes at the bottom of each figure, labeled to the left, with arrows indicating reading-frame direction (www.genome.ucsc.edu). Curly PWD photos courtesy of M. Bloom (Copyright AKC).

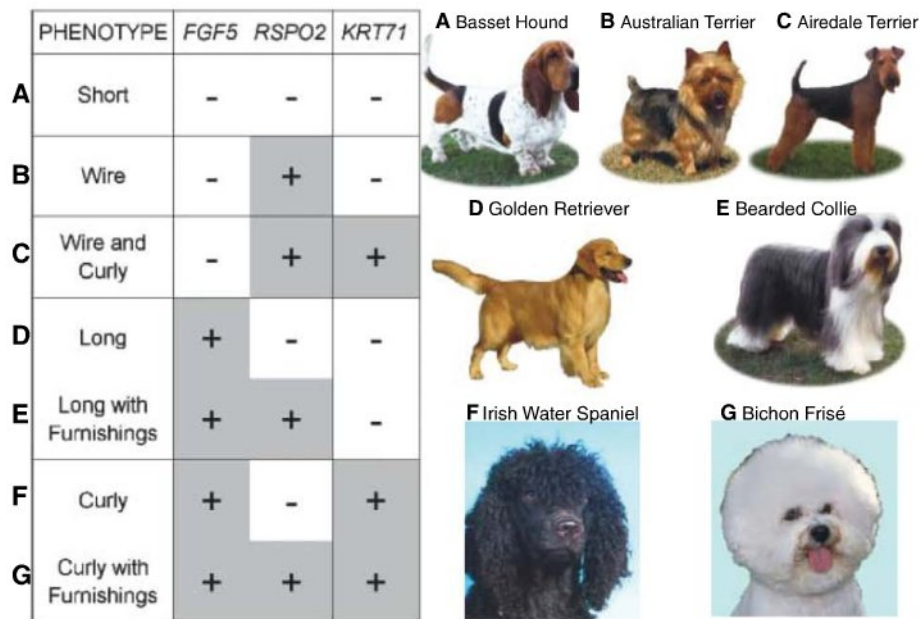


Fig. 3. Combinations of alleles at three genes create seven different coat phenotypes. Plus (+) and minus signs (–) indicate the presence or absence of variant (nonancestral) genotype. A characteristic breed is represented for each of the seven combinations observed in our data set: **(A)** short hair; **(B)** wire hair; **(C)** “curly-wire” hair; **(D)** long hair; **(E)** long, soft hair with furnishings; **(F)** long, curly hair; and **(G)** long, curly hair with furnishings. [Photos courtesy of M. Bloom (Copyright AKC)].

This diagnostic SNP was typed in several hundred additional dogs of varying hair length. Within the dachshunds, all long-haired dogs had the TT genotype, whereas all short or wire-haired dogs had either the GT or GG genotypes, suggesting a recessive mode of inheritance, as predicted previously (23). Across all breeds, the T allele was found in 91% of the long-haired dogs, in only 3.9% of the short-haired dogs, and accounts for ~30% of genotypes found in medium-haired dogs. Three breeds with very long hair, including the Afghan hound, neither carry the Cys⁹⁵→Phe variant nor show an association with CFA32, suggesting that additional loci exist that contribute to hair length in dogs (table S1).

To identify the gene that causes curly coat, we conducted a GWAS using PWDs (Fig. 2B) and identified a single associated SNP at position 5,444,030 on CFA27 ($P = 4.5 \times 10^{-7}$). A SNP in close proximity (5,466,995; $P = 6.9 \times 10^{-28}$) was associated with curly coat in the CanMap data set. Fine-mapping revealed a shared homozygous haplotype that included two keratin genes (Fig. 2C, fig. S4, and table S8). Sequence data covering 87% of the homozygous region identified one SNP at position 5,542,806 that segregated with the trait. Non-curly haired dogs carried the CC genotype; curly coated dogs had the TT genotype. In breeds where the trait segregates, such as PWDs, all three genotypes were observed. The relevant SNP is located in the *KRT71* gene (previously called K6irs1, Kb34, and K71) and causes a nonsynonymous Arg¹⁵¹→Trp alteration (table S9). Genotyping an additional 661 samples at this SNP validated the association ($P = 3 \times 10^{-92}$) (table S1).

Keratins are obvious candidates for hair growth [reviewed in (24)], and mutations in *KRT71* have been described in curly coated mice (25). The mutation described in our study is within the second exon of the gene and may affect either or both of two protein domains: a coiled-coil and a prefoldin domain (www.ensembl.org/Canis_familiaris/). Conceivably, sequence alterations in these domains could affect cellular targeting, receptor binding, or proper folding of the protein after translation [reviewed in (26)].

Notably, these three mutations in various combinations explain the observed pelage phenotype of 95% of dogs sampled, which include 108 of the ~160 American Kennel Club (AKC)-recognized breeds. A total of 622 dogs representing all identifiable coat phenotypes were genotyped at all three loci (table S10). By analyzing each of the three major traits both within and across multiple breeds, we show that combinations of these genotypes give rise to at least seven different coat types, encompassing most coat variation in modern domestic dogs (Fig. 3). Specifically, short-haired breeds display the ancestral state in all three genes. Wire-haired breeds, all of which have furnishings, carry the *RSPO2* insertion. Dogs that carry both the *RSPO2* and *KRT71* mutations display “curly-wire” hair that is similar in texture to wire-hair but longer

and curled or kinked rather than straight. Long-haired breeds carry the variant form of *FGF5*. Dogs carrying the *FGF5* mutation, along with the *RSPO2* insertion, have furnishings and long soft coats, rather than wiry ones. When dogs carry variants in both *FGF5* and *KRT71*, the pelage is long and curly. Not surprisingly, coats must be of sufficient length to curl, and all curly haired dogs in our study were homozygous for the *FGF5* mutation. Finally, if all three mutations are present, the phenotype is long and curly with furnishings.

None of the mutations we observed were found in three gray wolves or the short-haired dogs, indicating that short-haired dogs carry the ancestral alleles (table S1). Our finding of identical haplotypes surrounding the variants in all dogs displaying the same coat type suggests that a single mutation occurred for each trait and was transferred multiple times to different breeds through hybridization. Because most breeds likely originated within the past 200 years (27), our results demonstrate how a remarkable diversity of phenotypes can quickly be generated from simple genetic underpinnings. Consequently, in domesticated species, the appearance of phenotypic complexity can be created through combinations of genes of major effect, providing a pathway for rapid evolution that is unparalleled

in natural systems. We propose that in the wake of artificial selection, other complex phenotypes in the domestic dog will have similar tractable architectures that will provide a window through which we can view the evolution of mammalian form and function.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1177808/DC1

Materials and Methods

Figs. S1 to S5

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16 June 2009; accepted 14 August 2009

Published online 27 August 2009;

10.1126/science.1177808

Include this information when citing this paper.

JAK-STAT Signal Inhibition Regulates Competition in the *Drosophila* Testis Stem Cell Niche

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Adult stem cells often reside in local microenvironments, or niches. Although niches can contain multiple types of stem cells, the coordinate regulation of stem cell behavior is poorly understood. In the *Drosophila* testis, Janus kinase–signal transducer and activator of transcription (JAK-STAT) signaling is directly required for maintenance of the resident germline and somatic stem cells. We found that the JAK-STAT signaling target and inhibitor Suppressor of cytokine signaling 36E (SOCS36E) is required for germline stem cell maintenance. SOCS36E suppresses JAK-STAT signaling specifically in the somatic stem cells, preventing them from displacing neighboring germline stem cells in a manner that depends on the adhesion protein integrin. Thus, in niches housing multiple stem cell types, negative feedback loops can modulate signaling, preventing one stem cell population from outcompeting the other.

Adult stem cells reside in local microenvironments, or niches, in which signals from surrounding stromal cells inhibit differentiation (1). Niches are often structurally and molecularly complex, with diverse signaling pathways operating in a given niche (2). In niches containing multiple types of stem cells, it is unclear how behavior is coordinated to produce an appropriate ratio of differentiated cell types (3). Among the best-characterized niches are those in the *Drosophila* gonads, in which germline and

somatic stem cells cooperate during gametogenesis (3, 4). In the ovary, germline stem cells (GSCs) and somatic stem cells (called escort stem cells) share a niche. However, separate signals appear to regulate escort stem cell and GSC maintenance (5). In the testis, germline and somatic stem cells (cyst progenitor cells, or CPCs) reside in a single niche created by a small cluster of stromal cells (the hub) (fig. S1A), and both require Janus kinase–signal transducer and activator of transcription (JAK-STAT) signaling for their maintenance (6–8).

Thus, the *Drosophila* testis provides a tractable paradigm for understanding the coordinate regulation of multiple stem cell types within a single niche.

Because JAK-STAT signaling is directly required for GSC and CPC maintenance in the *Drosophila* testis (fig. S2 and table S1) (6–8), studying STAT targets should reveal regulatory mechanisms in one or both stem cell lineages. Transcriptional profiling identified putative STAT targets in this niche, including the JAK-STAT inhibitor Suppressor of cytokine signaling 36E (SOCS36E) (9). *socs36E* is expressed specifically at high levels in the hub and at lower levels in CPCs (fig. S1B) (9), making it an excellent candidate regulator within the testis niche.

SOCS proteins, first identified in vertebrates, are highly conserved antagonists of JAK-STAT signaling that act in a classic negative feedback loop: In response to signal activation, SOCS proteins bind and inhibit JAK kinases or their associated receptors so as to down-regulate the pathway

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intracellularly (10–13). By analyzing *socs36E* expression in testes with altered JAK-STAT signaling, we found that *socs36E* behaves as an induced antagonist within the *Drosophila* testis niche (fig. S1, C and D) (14).

To determine the role of *socs36E* in the niche, we identified a P-element insertion in the coding region of the *socs36E* gene. This insertion mutation, designated *socs36E^{PZ1647}*, creates a strong loss-of-function allele that interacts with JAK-STAT pathway components as a pathway antagonist (fig. S3) (14). *socs36E^{PZ1647}* flies are viable and fertile. Analysis by means of confocal microscopy, however, showed that the stem cell niche in *socs36E^{PZ1647}* testes from newly eclosed males is abnormal: It contains only three to four GSCs as compared with the wild-type average of 10 (Fig. 1, A to C). This phenotype is due to disruption of *socs36E* by the P-element because introducing a wild-type *socs36E* cDNA transgene into the *socs36E^{PZ1647}* mutant background rescues the phenotype, and precise excision of the P-element restores the number of GSCs to wild type. Moreover, the *socs36E^{PZ1647}* phenotype is due to loss, not gain, of *socs36E* function: *socs36E* heterozygotes have wild-type numbers of GSCs,

whereas testes containing *socs36E^{PZ1647}* in trans to a deletion of the *socs36E* locus phenocopy *socs36E^{PZ1647}*. Thus, *socs36E* is required for GSC maintenance in the testis.

Although the pool of GSCs is depleted in testes that lack *socs36E*, the positions typically occupied by these cells are filled with somatic cells (Fig. 1B). To identify these somatic cells we examined the expression of the zinc finger homeodomain-1 (ZFH-1) protein, which marks CPCs and their immediate cyst cell daughters (8). ZFH-1-positive nuclei are farther from the hub than GSC nuclei in wild-type testes but are often adjacent to the hub in *socs36E^{PZ1647}* testes (Fig. 1, D and E). Ultrastructural analysis confirmed that CPCs in wild-type testes display a small region of contact with the hub, whereas GSCs maintain large regions of contact (15); but in *socs36E* testes, the situation is reversed: CPCs form aberrantly broad contacts with the hub (Fig. 1, F and G). We considered that the decreased pool of GSCs in *socs36E^{PZ1647}* testes could be accompanied by a corresponding increase in the number of CPCs. However, the number of ZFH-1-positive cells was the same in *socs36E^{PZ1647}* and wild-type testes (34.8 ± 1.6 , $n = 17$ testes, versus 35.7 ± 1.6 ,

$n = 17$ testes, $P > 0.05$), and these cells displayed similar rates of proliferation (measured by mitotic ZFH-1-positive cells per testis 0.4 ± 0.1 , $n = 22$ testes, versus 0.5 ± 0.1 , $n = 50$ testes, $P > 0.05$). Thus, the GSC loss and concomitant expansion of CPC/hub cell contacts in *socs36E^{PZ1647}* testes is not due to CPC overproliferation.

Because both GSCs and CPCs respond directly to JAK-STAT signaling, *socs36E* could be directly or indirectly required to prevent GSC loss. To distinguish between these possibilities, we created *socs36E* loss-of-function clones (14) and analyzed the effects on each lineage. This analysis revealed that GSCs do not directly require *socs36E* for their maintenance (Fig. 2, A and B, and table S2). In contrast, we uncovered a role for *socs36E* in CPCs. Testes initially containing only a few mutant CPCs gain significantly more mutant CPCs over time than those of controls, indicating that *socs36E* prevents CPCs from accumulating within the niche (Fig. 2, C and D, and table S2). Over the time course of this experiment, the total number of CPCs remains constant, but the number of mutant CPCs increases, indicating that CPCs lacking *socs36E* outcompete wild-type CPCs (table S2).

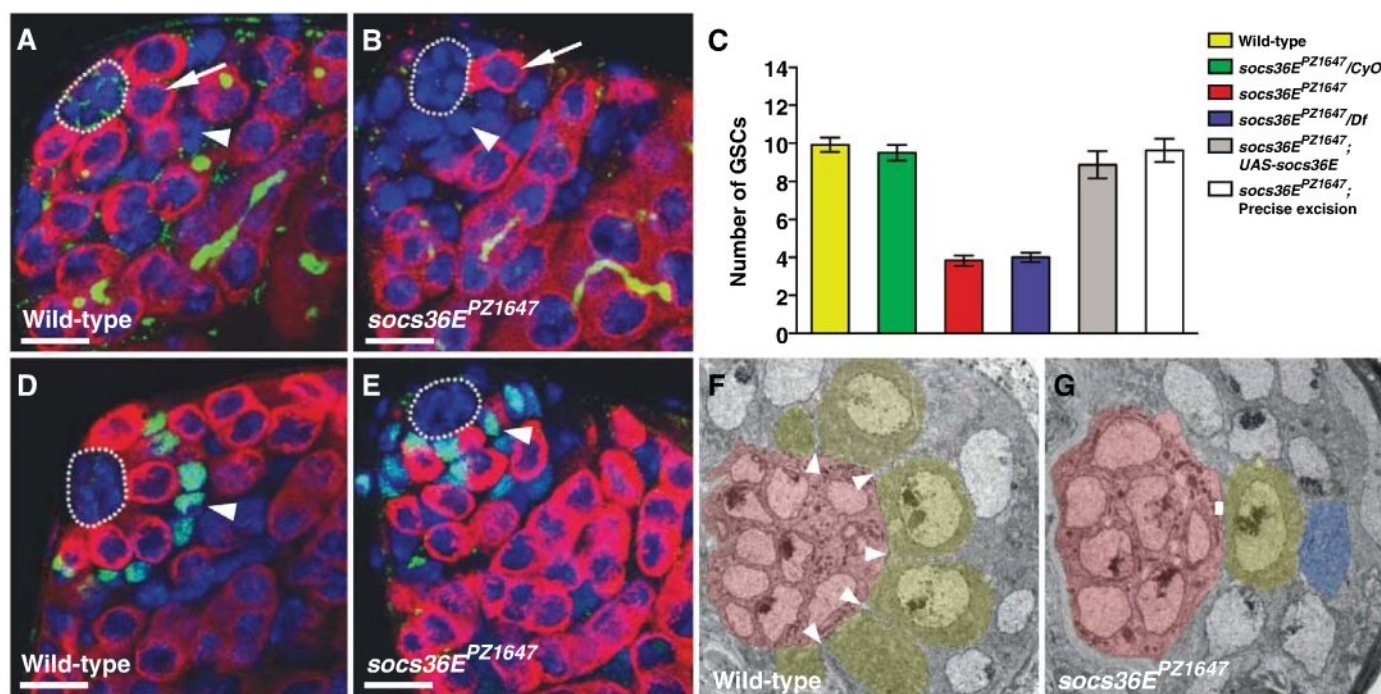


Fig. 1. *socs36E* is required to maintain germline stem cells. (A to E) Confocal section through the testis apex immunostained with [(A) and (B)] antibody to Vasa (germline, red), antibody to Fasciclin III (hub, green), antibody to Hts (1B1 fusome, green), and 4',6'-diamidino-2-phenylindole (DAPI) (DNA, blue) or with [(D) and (E)] antibody to Vasa (red), antibody to ZFH-1 (CPCs and their immediate daughters, green), and DAPI (blue). The hub is outlined. (A) In wild-type testes, a rosette of GSCs (one indicated, arrow) surrounds the hub; Vasa-negative CPCs (one indicated, arrowhead) make minimal contact with the hub. (B) *socs36E^{PZ1647}* testes have fewer GSCs (arrow); instead, somatic cells (arrowhead) surround the hub. (C) Bar graph showing number of GSCs per testis. Homozygous mutant *socs36E^{PZ1647}* testes contain significantly fewer GSCs than do wild-type (red, 3.8 ± 0.2 , $n = 12$ testes, versus yellow, 9.9 ± 0.4 , $n = 14$ testes, $P < 0.0001$) or *socs36E* heterozygotes (green, 9.5 ± 0.4 , $n = 14$ testes).

Testes from *socs36E^{PZ1647}* in trans to a deletion of *socs36E* (*Df(2L)M36F-S5*) phenocopy *socs36E^{PZ1647}* (blue, 4.0 ± 0.2 , $n = 12$ testes). Leaky expression from a *socs36E* cDNA transgene rescues the phenotype (gray, 8.9 ± 0.7 GSCs, $n = 15$ testes). Precise excision of the P-element restores GSC number to wild-type (white, 9.6 ± 0.6 , $n = 11$ testes). (D) In wild-type testes, GSCs surround the hub; nuclei of ZFH-1-positive CPCs (arrowhead) are displaced from the hub. (E) In *socs36E^{PZ1647}*, CPCs (arrowhead) occupy positions at the hub that usually contain GSCs. (F and G) Ultrastructural analysis of the testis niche. Hub cells (red), GSCs (yellow), and a gonialblast (blue), identified by differences in morphology and staining density (29), are indicated. (F) In wild-type testes, GSCs make broad contact with the hub; CPCs extend thin projections (arrowheads) between GSCs. (G) In *socs36E^{PZ1647}*, CPCs broadly contact the hub, whereas GSCs make small areas of contact with the hub (rectangle). Scale bars, 10 μ m.

The advantage for niche occupancy that mutant CPCs display over their wild-type counterparts even extends to GSCs: As the number of mutant CPCs increases, there is a corresponding decline in the number of GSCs (table S2). Even small clones of CPCs lacking *socs36E* recapitulate the *socs36E^{PZ1647}* phenotype. Thus, our clonal analysis indicates that *socs36E* is not needed in GSCs to prevent them from “losing their grip” and leaving the niche. Instead, *socs36E* acts directly in CPCs to prevent them from displacing GSCs.

These data suggest that inhibition of JAK-STAT signaling via *socs36E* within CPCs maintains the proper ratio of germline and somatic stem cells within the niche. We assessed the distribution of Stat92E protein in testes (16), which reflects JAK-STAT signaling activity and thus probably reflects the limits of the niche (17–19). In wild-type testes, Stat92E is highly enriched in GSCs but rapidly declines in their differentiating daughters. CPCs and hub cells contain relatively little Stat92E, suggesting that GSCs normally have higher levels of pathway activation than do CPCs (Fig. 2E) (20). In contrast, hub cells and CPCs contain elevated levels of Stat92E in *socs36E^{PZ1647}* testes (Fig. 2F). We conclude that *socs36E* normally attenuates JAK-STAT signaling in hub cells and CPCs, maintaining the appropriate balance of the two stem cell lineages in this niche.

We considered that *socs36E* could regulate competition between GSCs and CPCs by regulating adhesion and/or signaling within the niche. Although cadherin-mediated adhesion sustains stem cell maintenance in this and other niches (20, 21), we found no discernible differences in the expression patterns of DE- or N-cadherin between wild-type and *socs36E* mutant testes (fig. S4). In contrast, *socs36E^{PZ1647}* testes have elevated levels of the common beta subunit of position-specific integrins (β PS-integrin), particularly at the CPC-hub cell interface (Fig. 3, A and B), suggesting that integrin-mediated adhesion plays a role in maintaining the correct GSC-to-CPC ratio. To provide genetic evidence for this hypothesis, we asked if reducing integrin function reverses the GSC loss phenotype in *socs36E^{PZ1647}* testes. Removing one copy of *rhea*, which encodes *Drosophila* talin, an integrin-binding cytoskeletal linker essential for integrin-mediated adhesion (14, 22), did not cause GSC loss. However, this level of reduction in integrin function was sufficient to rescue the *socs36E^{PZ1647}* phenotype (Fig. 3C), indicating that SOCS36E normally prevents CPCs from outcompeting GSCs from the niche via an integrin-mediated mechanism.

These data suggest that CPCs with aberrantly high levels of JAK-STAT signaling have increased

integrin-mediated adhesion to the hub and therefore preferentially gain niche occupancy. Thus, increasing the level of β PS-integrin in CPCs may enable them to outcompete neighboring wild-type GSCs. Consistent with this hypothesis, overexpression of β PS-integrin in CPCs and cyst cells reduced the number of GSCs per testis significantly (11.3 ± 0.4 , $n = 10$ testes, versus 7.5 ± 0.2 , $n = 30$ testes, $P < 0.0001$) (Fig. 3D). Overexpression of β PS-integrin in CPCs and cyst cells did not significantly alter their numbers (Fig. 3D) or rate of proliferation (measured by mitotic ZFH-1-positive cells per testis 0.4 ± 0.1 , $n = 24$ testes, versus 0.7 ± 0.2 , $n = 33$ testes, $P > 0.05$). Thus, as in *socs36E^{PZ1647}* testes, CPCs with ectopic β PS-integrin gain an advantage for niche occupancy that is not driven by overproliferation. Together, these data indicate that increased expression of integrin in CPCs leads to their enhanced adhesion to the hub, enabling them to outcompete GSCs.

We propose that *socs36E* acts in a negative feedback loop to inhibit JAK-STAT signaling in CPCs, ensuring that the proper balance of germline and somatic stem cells is maintained in the niche. Although JAK-STAT is directly activated in both stem cell lineages, *socs36E* expression in CPCs prevents them from displacing GSCs, most likely by limiting integrin-mediated adhesion of CPCs to the hub. Although our work highlights

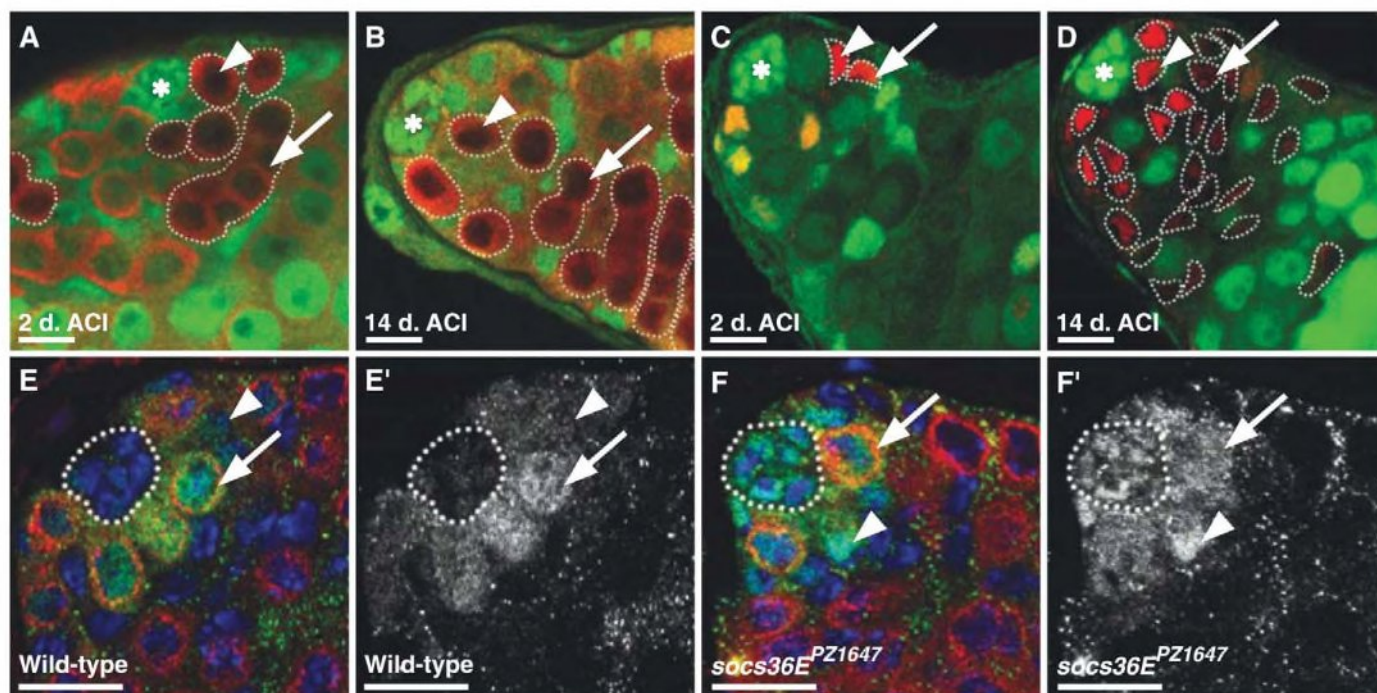
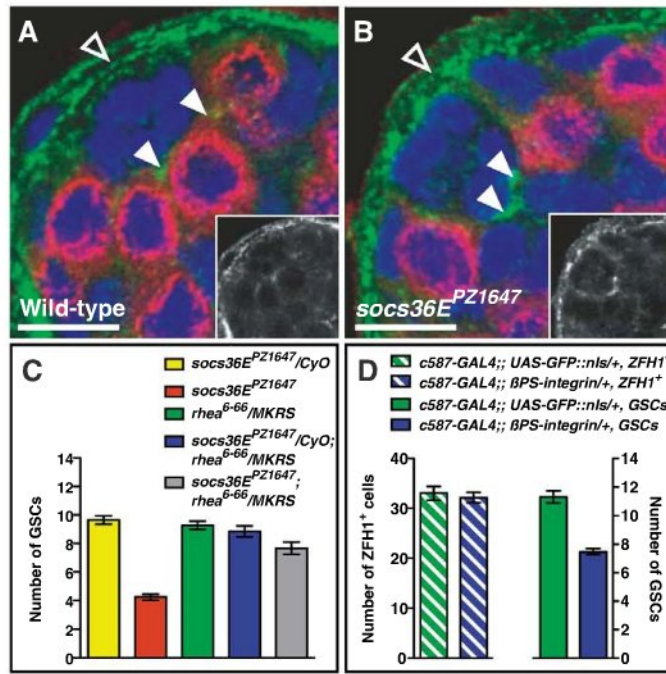


Fig. 2. *socs36E*-mediated JAK-STAT pathway inhibition prevents CPCs from outcompeting neighboring GSCs. [(A) to (F)] Confocal sections through the testis apex. [(A) to (D)] Hubs denoted by asterisks. [(A) and (B)] *socs36E^{PZ1647}* GSC clones (outlined, arrowhead) and their differentiating progeny (outlined, arrow) identified by the absence of green fluorescent protein (GFP) (green) and the presence of Vasa (red) at (A) 2 days and (B) 14 days after clonal induction (ACI). GSCs at the hub and their progeny are maintained. [(C) and (D)] *socs36E^{PZ1647}* CPC clones (outlined, arrowhead) and their differentiating daughters (outlined, arrow) identified by the absence of

GFP (green) and the presence of ZFH-1 (red) at (C) 2 days and (D) 14 days ACI. CPCs lacking *socs36E* accumulate around the hub, competing for niche occupancy and leading to a loss of GSCs over time. [(E) and (F)] Testes immunostained with antibody to Stat92E (green), antibody to Vasa (red), and DAPI (blue); GSCs (arrows), CPCs (arrowheads), and hubs (outlined) are indicated. [(E') and (F')] Stat92E channel. [(E) and (E')] In wild-type testes, Stat92E is detectable at higher levels in GSCs than in CPCs or the hub. [(F) and (F')] In *socs36E^{PZ1647}* testes, Stat92E levels in hub cells and CPCs are increased relative to wild-type controls. Scale bars, 10 μ m.

Fig. 3. Integrins mediate competition between germline and somatic stem cells in the testis niche. (A and B) Confocal section through the testis apex immunostained with antibody to Vasa (red), antibody to β PS-integrin (green, and in insets), and DAPI (blue). (A) In wild-type testes, β PS-integrin is expressed strongly in the sheath (open arrowhead) and at moderate levels throughout the apex; within the niche, it is enriched at points of CPC-hub cell contact (arrowheads). (B) In *socs36E^{PZ1647}* testes, expression of β PS-integrin at the CPC-hub interface (arrowheads) is increased relative to wild-type controls. (C) *socs36E^{PZ1647}*



GSC loss is partially rescued by introducing one copy of a *talpin* loss-of-function allele (*rhea⁶⁻⁶⁶*) (red, 4.2 ± 0.2 , $n = 24$ testes, versus gray, 7.7 ± 0.4 , $n = 18$ testes, $P < 0.0001$). Testes from control flies (*socs36E^{PZ1647}* or *rhea⁶⁻⁶⁶* heterozygotes and *socs36E^{PZ1647}; rhea⁶⁻⁶⁶* transheterozygotes) contain wild-type numbers of GSCs (yellow, 9.6 ± 0.3 , $n = 14$ testes; green, 9.3 ± 0.2 , $n = 22$ testes; and blue, 8.8 ± 0.3 , $n = 24$ testes, respectively). CyO and MKRS denote balancer chromosomes containing wild-type alleles of *socs36E* and *rhea*. (D) Expression of ectopic β PS-integrin in CPCs and cyst cells significantly reduces the number of GSCs per testis as compared with *UAS-GFP* controls. Scale bars, 10 μ m.

the importance of adhesion in competition between these two distinct stem cell lineages, it is also possible that changes in cell signaling occur when *socs36E* is disrupted. For example, because the epidermal growth factor receptor (EGFR) pathway acts within the CPC lineage to promote GSC differentiation (23, 24), and *socs36E* can inhibit both EGFR and JAK-STAT signaling (11), *socs36E* might interact with additional signaling pathways within the niche.

Our work exemplifies how one signaling pathway coordinately maintains two types of stem cells in a single niche. Furthermore, it underscores the importance of feedback inhibition at the cellular and molecular levels within stem cell niches. Both of these features may emerge as common themes

of mammalian niches as they become better characterized. Both aberrant JAK-STAT signaling (including the loss of SOCS gene expression) and disrupted integrin function can lead to tumorigenesis in mammals (25–27). Because misregulation of stem cells is implicated in many types of cancer (28), it will be important to determine whether altered JAK-STAT signaling and integrin-mediated adhesion enhance the ability of additional types of cells to outcompete normal stem cells so as to establish occupancy of stem cell niches.

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Supporting Online Material

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26 May 2009; accepted 13 August 2009

10.1126/science.1176817

INNOVATION MORE CRITICAL THAN EVER

The year 2009 has been a time of change for the biotechnology and pharmaceutical industry. With a weakened global economy and dwindling drug pipelines, many of the companies that made the list of *Science*'s 2009 top employers are finding that maintaining innovation through collaborations and partnerships is critical to success. **By Laura Bonetta**

The global economic meltdown severely damaged many industries. Although the pharmaceutical industry is normally relatively immune to economic downturns, most biotechnology and pharmaceutical companies have had to find ways to tighten their belts. In addition, for many of them the pipeline of promising drug candidates has dried up at around the same time that existing drugs are set to lose their patent protection. To feed pipelines and cut costs some companies have merged, while many others have formed partnerships.

The shifting political landscape has also brought change. In the United States, the ongoing push for health care reform may mean that drugs will soon be marketed differently. And heightened public concern about drug safety has resulted in new legislation that will lead to more stringent safety regulations by the US Food and Drug Administration (FDA).

But not everything is changing. For one thing Genentech, now a wholly owned member of the Roche group, has not budged from its No. 1 position in this year's *Science* survey of top employers in the industry. "We are delighted to once again be named top employer by *Science* magazine," says **Marc Tessier-Lavigne**, Genentech's executive vice president for research and chief scientific officer. "The honor is a real testament to Genentech's unique culture and to the dedication of our employees." The company held on to the No. 1 spot despite the fact that at about the same time the web-based survey was being completed in March 2009, Genentech was acquired by the Swiss company Roche.

The over 2,000 people who responded to the top employer survey selected companies, including their own, that they regarded as best, average, and worst employers. And although they shuffled places somewhat, most of the companies who made it to the top 20 list (see figure on p. 162) were on that list last year and the year before that. One exception is Syngenta. A company providing seeds and crop protection products, Syngenta made the list for the first time this year at No. 20.

Between Genentech and Syngenta, the top 20 employers include Boehringer Ingelheim at No. 2, followed by Genzyme and then Monsanto. Millennium: The Takeda Oncology Company, which took its new name after Millennium was acquired by Takeda Pharmaceuticals in May 2008, came in at No. 5, followed by Merck KGaA, Schering-Plough, Amgen, Gilead Sciences, and Johnson & Johnson at No. 10. Next came Eli Lilly and Company, Novartis, GlaxoSmithKline, AstraZeneca, Biogen Idec, Merck, Roche, Wyeth, and sanofi aventis. Among these top 20 companies, 12 are headquartered in the United States and eight are in Europe.

As in previous years, all survey takers rated the companies they had chosen on 23 driving characteristics. *Being an innovative leader in the industry* remains the most powerful driving characteristic of top employers. In addition, *doing important, quality research, being socially responsible with loyal employees, having leaders who keep the organization moving in the right direction, having values that are aligned with employees' personal values, and treating employees with respect* are the other important drivers selecting the best company.

All companies on the top 20 list received high marks for these drivers, with some companies doing better on some drivers than others (see figure on p. 168).

Innovation Is Key

Being a leader in new discoveries and innovation is what many of the companies on the top 20 list strive to achieve. For three consecutive years, this driver was the top criterion among survey takers in selecting the best company to work for. **continued »**



“Being a leader in new discoveries and innovation is what many of the companies on the top 20 list strive to achieve.”

UPCOMING FEATURES

Careers in Neuroscience — October 16

Jobs in Government — October 23

Reentering the Work Force — November 27

TOP TWENTY EMPLOYERS



2009 Rank	2008 Rank	Employer (Global Headquarters)	Innovative leader in the industry	Treats employees with respect	Has loyal employees	Is socially responsible	Does important	Leadership moves company in right direction	Work and personal values are aligned
1	1	Genentech, Inc. (South San Francisco, CA)	•	•	•	•	•	•	•
2	4	Boehringer Ingelheim (Ingelheim, Germany)	•	•	•	•	•	•	•
3	3	Genzyme Corp. (Cambridge, MA)	•	•	•	•	•	•	•
4	2	Monsanto (Creve Coeur, MO)	•	•	•	•	•	•	•
5	8	Millennium: The Takeda Oncology Co. (Cambridge, MA)	•	•	•	•	•	•	•
6	--	Merck KGaA (Darmstadt, Germany)	•	•	•	•	•	•	•
7	5	Schering-Plough Corporation (Kenilworth, NJ)	•	•	•	•	•	•	•
8	20	Amgen (Thousand Oaks, CA)	•	•	•	•	•	•	•
9	9	Gilead Sciences (Foster City, CA)	•	•	•	•	•	•	•
10	15	Johnson & Johnson (New Brunswick, NJ)	•	•	•	•	•	•	•
11	10	Eli Lilly and Company (Indianapolis, IN)	•	•	•	•	•	•	•
12	11	Novartis (Basel, Switzerland)	•	•	•	•	•	•	•
13	19	GlaxoSmithKline (London, UK)	•	•	•	•	•	•	•
14	12	AstraZeneca PLC (London, UK)	•	•	•	•	•	•	•
15	18	Biogen Idec (Cambridge, MA)	•	•	•	•	•	•	•
16	14	Merck & Co., Inc. (Whitehouse Station, NJ)	•	•	•	•	•	•	•
17	6	Roche (Basel, Switzerland)	•	•	•	•	•	•	•
18	13	Wyeth (Madison, NJ)	•	•	•	•	•	•	•
19	16	sanofi-aventis (Paris, France)	•	•	•	•	•	•	•
20	--	Syngenta (Basel, Switzerland)	•	•	•	•	•	•	•

Survey Methodology

E-mail invitations to take a web-based survey were sent to roughly 75,000 individuals worldwide, who were AAAS members, *Science* Careers registrants, *Science* website visitors, and past survey takers. E-mails were also sent to approximately one thousand human resources contacts at industry firms. A total of 2,334 people responded by taking the survey between March 3 and 28, 2009; 73 percent from North America, 15 percent from Europe, 8 percent in the Asia/Pacific Rim. Survey participants were asked to write in the names of the companies that they regarded as best, average, and worst employers. In total 575 companies were cited. Survey takers then rated the companies they had chosen on 23 driving characteristics. The top 20 companies were selected using a statistical process that calculates a unique ranking score for each company. Only companies that were rated by 30 or more respondents were eligible to become part of the top 20 employers. Fifty-eight percent of respondents chose as best employer the company they were currently employed by, whereas 18 percent chose companies that they knew through colleagues or friends and 14 percent selected a company to which they had no relationship.

The 20 companies with the best reputations as employers and the top three driving characteristics for each company, according to respondents in the 2009 survey undertaken for the *Science*/AAAS Business Office. The companies without a 2008 rank did not receive enough mentions to qualify during the 2008 survey.

At Amgen “we look for approaches to disease that have never been pursued,” says **Roger Perlmutter**, executive vice president for research and development. “We avoid me-too research.” A recent example of innovative science is the development of an antibody that targets a protein secreted by bone cells and that inhibits bone formation. That research, according to Perlmutter, has revealed fundamental insights into bone biology, as well as being developed as a possible treatment for bone diseases.

“We don’t do work where someone has already pursued a target and we can make another version of that drug,” says Perlmutter. “That is not to say that kind of work is unimportant. Changing a drug so that it can be taken once a day rather than twice a day makes a big difference in a patient’s life. But it’s not what we do.”

The focus on innovation is just as important for other types of biotechnology companies, even ones that are not in the business of making drugs. “Over the course of time we have proven that we are leaders in innovation,” says **Roger Kemble**, head of crop genetics research and interim president of Syngenta.

(Last year, for the first time ever, a company outside the biopharmaceutical business reached the first tier of top employers—2008

survey respondents voted Monsanto into second place after Genentech. This year Monsanto dropped to fourth place, but Syngenta, another company that applies biotechnology and traditional chemistry to the development of agricultural products, made the list of top employers for the first time.)

“We pursue innovative ideas that did not exist before. We are not improving something that existed. We are starting with a clean slate, using sophisticated tools and talented people to bring plant potential to life,” says Kemble. “Innovation comes from realizing that research can only advance if we have innovative people to advance it and if we give them time and space to innovate.”

One way in which the company rewards innovation is through an internal awards program. “It’s a bit like the Oscars for us,” says Kemble. Syngenta employees who had innovative ideas that were later put into practice submit written descriptions of their projects. A special committee then selects the best projects. In 2008, the program had record participation, with around a thousand projects submitted, representing 9,400 employees around the world, about two-thirds of the total work force. **continued »**



Demographics

Gender

57% male, 43% female

Experience

59% have 10 years or more work experience

Career Status

75% report that they have not yet reached the peaks of their careers

Company Type

51% biotech, 25% pharma, 13% university; more than 4 out of 5 work in private industry

Geography

73% from North America; 15% from Europe; 8% from Asia/Pacific Rim

Maintaining the Edge

Given the recent economic turmoil and the fact that patent protection on many successful drugs is set to expire in a few years, maintaining innovation is more essential than ever to a company's survival. And each company has gone about increasing and maintaining innovation through different approaches.

"We have a new CEO, Andrew Witty, who has been incredibly dynamic in helping the company understand that the old pharma model has to change," says **Nigel Broom** director of UK R&D recruitment at GlaxoSmithKline (GSK), the largest pharmaceutical company in the UK. "And that we will be leaders and not followers in that change."

To increase its early pipeline, GSK has shifted focus to scientific areas that are most likely to lead to new medicines. Together with vaccines, GSK's R&D is now focused on biopharmaceuticals, immunoinflammation, infectious diseases, metabolic pathways, neuroscience, oncology, ophthalmology, and respiratory conditions. "We have been making the change for a while, certainly in R&D," says Broom.

In addition, the company has expanded collaborations with external partners as well as with academia. Recent alliances include organizations such as Cellzome and the Harvard Stem Cell Institute, as well as the acquisition of small biotech companies like Sirtris and Genelabs.

The year 2008 saw the creation of discovery performance units (DPUs) within GSK. Each DPU is a compact, fully empowered, focused, and integrated team which has responsibility for a small part of the pipeline. "Each DPU has guaranteed funding for three years and then the funding is reassessed," says Broom. "They are run as mini businesses and people are very engaged. They have to regularly justify why they need continued funding." These units, which had been running for about six to nine months by the time this article went to press, are "reflective of the changes that are going on at GSK," says Broom.

GSK monitors the level of satisfaction among its employees on a regular basis through a survey aptly called PULSE. "We have done a fair bit of changing. At first people were uncertain about it, but the approval numbers have now bounced back and gotten higher," says Broom. "There is enthusiasm for the work and a commitment to it. There is a lot of commitment to Witty and the path he is taking us."

The Importance of Research

One of the key ingredients of innovation is cutting-edge research. This year's survey respondents identified research quality as one of the top six drivers of the best employers—a characteristic that was not on the list last year (see figure on p. 166).

Over 90 percent of survey takers who selected the top four employers (Genentech, Boehringer Ingelheim, Genzyme, and Monsanto) agreed that these companies engage in important, high-quality research. In addition, doing high quality research was among the top three drivers for 18 employers out of the top 20 (see figure on p. 162).

"Genentech was founded on doing great science, with a focus on translational research," says Genentech's Tessier-Lavigne. Genentech's co-founder, Robert Swanson, who died in 1999, often referred to the importance of Genentech's employees by saying, "our most valuable assets go home every evening in tennis shoes," recalls Tessier-Lavigne.

Tessier-Lavigne is one of several established scientists who were brought to Genentech's top management from academia. Because of its focus on high-quality research and informal atmosphere, Genentech has long been viewed as a cross between academia and industry. Vishva Dixit, vice president of physiological chemistry, joined Genentech in 1997 from the University of Michigan. "When people ask him 'What is it like working in industry?' he answers 'I don't know, I went to Genentech,'" says Tessier-Lavigne.

Genentech's employees value "being part of a science-driven culture and one with very little hierarchy," he adds. "But what inspires them is being focused on improving patients' lives. That's what motivates us here."

Flat Hierarchies

It comes as no surprise that scientists don't like hierarchy and most companies that made the top 20 list understand that. Representatives from these companies typically describe their company's environment as being open and with little top-to-bottom decision making.

"Scientists live for initiatives, they live for motivation. They need to be taken seriously and not micromanaged," says **Gerd Schnorrenberg**, senior vice president for research for Boehringer Ingelheim, based at the company's largest research site in Biberach, Germany. "We have very flat hierarchy in research. Our scientists report their project results in management meetings directly to board members."

With 41,300 employees worldwide, Boehringer Ingelheim has 1,700 researchers (out of a total of 6,700 employees in the whole research and development area). A relatively small research group helps maintain informal and open communication. "Among us researchers, we all know each other quite well," says Schnorrenberg.

The company has performed several so-called "landmark" studies. The results of one study, known as UPLIFT, for example, one of four large-scale studies conducted in 2008, demonstrated the effectiveness and safety of a drug called Spiriva in about 6,000 patients with respiratory disease.

Because the company is privately owned, "we are able to invest in mega trials without any concerns about how Wall Street will react," says **David Nurnberger**, senior vice president, human resources at Boehringer Ingelheim Pharmaceuticals, the company's US affiliate. "We have no public stock, so we can maintain a long-term view. We can invest in large, sometimes long, trials to get the data we need." **continued »**



Driving Characteristics of Top Employers

2009

1. Innovative leader in the industry
2. Treats employees with respect
3. Loyal employees
4. Socially responsible
5. Does important, quality research
6. Leadership moves company in right direction
7. Work and personal values are aligned

2008

1. Innovative leader in the industry
2. Leadership moves company in right direction
3. Treats employees with respect
4. Loyal employees
5. Socially responsible
6. Work and personal values are aligned

Black type indicates the characteristics in common for the two years.

Valuing Employees

In addition to a commitment to research and little hierarchy, other characteristics common to companies in the list of top 20 employers include *respecting employees* and *having loyal employees*.

"We have roundtable discussions where executives, including our CEO, meet with a random group of 10 employees at a time and openly respond to whatever the employees want to talk about," says **Craig Schneider**, executive vice president of human resources at Biogen Idec. "They are very candid exchanges."

A company of about 4,700 employees, Biogen Idec has been conducting these roundtable discussions for several years, tapping about a thousand employees every year. "Our employees express a lot of confidence and support for leadership and part of the reason is that we actively communicate with them," says Schneider.

Another important criterion for employers is working for a company with values that are aligned to the employee's own. At Johnson & Johnson the values that guide decision making are spelled out in the company's Credo, according to **Anuk Das**, assistant director of immunobiology at Centocor R&D, a Philadelphia-based subsidiary of Johnson & Johnson.

Robert Wood Johnson, former chairman from 1932 to 1963 and a member of the company's founding family, crafted the Credo in 1943, just before Johnson & Johnson became a publicly traded company. "What is impressive is that the Credo is still carried out today," says Das.

Johnson & Johnson's Credo lists the company's four priorities in order of importance: patients and their families, employees, communities, and stockholders. "For me my loyalty for Johnson & Johnson comes from the Credo and the demonstration that our leaders use it as a guide to make decisions," says Das.

Mergers and Acquisitions

It may be challenging for a company to do right by its employees at the time of a merger or acquisition. Such steps are often accompanied by layoffs, in some cases leading to the loss of thousands of jobs. On the upside, survey respondents pointed out that mergers can provide a company with access to a pipeline of new products and the chance to cut costs, ultimately benefiting employees.

This year has seen many high-profile mergers and acquisitions. In January 2009 New York-based Pfizer, maker of the blockbuster drug Lipitor, which loses patent protection in November 2011, announced

plans to buy Wyeth. In March, Merck & Co. bought Schering-Plough Corporation (both companies made the list of top employers at positions 16 and 7, respectively).

In March 2009 Genentech became a wholly owned member of the Roche group, headquartered in Basel, Switzerland. Under the new organizational structure, Genentech Research and Early Development program, dubbed gRED, which essentially comprises basic research up

to Phase 2 clinical trials, will maintain its autonomy. "The leadership at Roche knew the strength of our research program and wanted to maintain what we had developed here," says Tessier-Lavigne. "They have left us independent, so we have been able to maintain our research culture without missing a beat."

The merger did of course cause some changes. Arthur Levinson, Genentech's former chair and CEO, and Susan Desmond-Hellmann, former president of product development, both left the company. (In May 2009, Desmond-Hellmann was named chancellor of the University of California, San Francisco.) But as far as Genentech's research focus and unique culture—which includes a vibrant postdoctoral program as well as free cappuccinos and Friday evening parties—are concerned, those will not change, says Tessier-Lavigne. "We have maintained our momentum throughout the merger and now we are moving on the next exciting chapter in our company," he says.

Is a smooth takeover really possible? Apparently yes, according to **Joe Bolen**, chief scientific officer at Millennium. Established in 1993 as a genomics company, Millennium has since grown into a fully integrated biopharmaceutical company. In May 2008 the company was acquired by Takeda Pharmaceutical Company Limited, the largest pharmaceutical company in Japan. Millennium, now renamed Millennium: The Takeda Oncology Company, operates as an independent subsidiary serving as Takeda's global center of excellence in oncology.

Under Takeda, Millennium has been involved in a larger number of clinical trials and has been testing new compounds obtained from its parent company. "But the philosophy of the earlier research and development has not changed," says Bolen. "We worked hard when Millennium was first set up to figure out the best things to do. We worked out procedures to make early-phase research as seamless as could be. Under Takeda, we were able to continue the same processes and maintain our culture and focus."

The strategy appears to be paying off. In April 2009 the company published an article describing a new molecule that modulates the levels of proteins critical to the regulation of cancer cell growth and survival. The compound has now moved forward to Phase 1 studies. **continued »**

You can find an expanded version of this feature by going to:
dx.doi.org/10.1126/science.opms.r0900079

"We were reassured we would maintain our identity and independence and I am happy to say that the president of Takeda, Yasuchika Hasegawa, kept his word and allowed us to flourish," says Bolen. "Most takeovers are not happy, but this is an acquisition that will be a success story." Perhaps that is why, after the acquisition, Millennium has moved to fifth place in the top employers survey, up from No. 8 in 2008 and 13 in 2007.

Innovation Through Collaboration

Some companies have made it part of their mission to keep

away from mergers and acquisitions. "We are a very independent company and always plan to be that way," says **Rich Gregory**, Genzyme's senior vice president and head of research. "The acquisitions within the pharma industry are symptomatic of many companies with relatively weak pipelines that are acquiring innovation by consolidating with larger companies to reduce expenses." But Genzyme has taken the approach to invest in research internally, as well as establishing numerous collaborations with other companies and academic researchers, explains Gregory.

For example a collaboration between Genzyme and PTC Therapeutics led to experimental new drugs with potential to address many devastating genetic diseases, including Duchenne muscular dystrophy and cystic fibrosis. "Those drugs are based on an entirely novel technology that we acquired through collaboration," says Gregory.

Being able to acquire new technologies, as well as becoming more innovative internally by venturing into new research areas, such as stem cell and gene therapy research, have allowed Genzyme to maintain its edge. "We evolve constantly. We are not a static company, but will change as the business changes," says Gregory. "At the same time, our employees value working for a stable company."

Indeed a common theme for employees at top-tier biotechnology or pharmaceutical companies is the opportunity to collaborate with a wide range of researchers from different backgrounds and get exposed to different technologies and fields of research. "We are constantly innovating. It is probably a lot more fun for employees," says Gregory.

Stable Environments

Boehringer Ingelheim, a privately held company, is among the ones committed to independence. "You have to find the right balance between continuity and change. We see the necessity to change and to adapt to future challenges," says Schnorrenberg. "One way to cope is not to merge, but to collaborate with academic labs and startups."

The strategy has helped the company be financially successful, according to Schnorrenberg. "We increased our R&D budget by 22 percent in 2008. Not too many companies have been able to do that," he says. "Part of the reason is that we have not been saddled with all the costs that go with mergers and acquisitions."



Comparison of the top 10 companies on the basis of the top 3 drivers (scored out of 100): Loyalty (bubble width), Innovative (x-axis), and Treats employees with respect (y-axis).

The strategy is also reassuring to employees. "Our employees are not worried about the company being acquired; that is a really important facet of working for Boehringer Ingelheim," says Nurnberger. "We receive many applications from people from other companies who complain about constant changes in direction and management. We are attractive to talented individuals looking for a stimulating work environment in a privately owned company."

This year's survey identified that there were slightly more job seekers in the market (30 percent compared to 27 percent in 2008). Fewer people this year were looking for a new job for career advancement than last year, but more are moving because of "company direction" and for reasons related to mergers and acquisitions or expected layoffs that were taking place at the survey taker's company.

Working for the Greater Good

Job insecurity due to mergers and acquisitions, as well as some reprioritization and downsizing due to the global economic meltdown, was identified as one of the two disadvantages of working in the biotech and pharmaceutical industry by survey takers. The other disadvantage is the negative public image perception due to withdrawn products and safety issues.

"Any time you are dealing with powerful medicines, you have the potential issue of safety. As a company we make sure we do everything above board and carefully," says Biogen Idec's Schneier. "Employees want to know that the company they work for is ethical and transparent and has done all the right things."

But survey participants identified many advantages of working for the biotech and pharmaceutical industry. They include engaging work and being part of cutting-edge research and development; innovative, creative, and fast-paced work; and stability in difficult economic times, including generous salaries, benefits, and job security.

And despite all the challenges, one of the main advantages of working in this industry is its mission: advancing the health of people and working for the greater good. "What I focus on is the reason why I chose to be a scientist," says Johnson & Johnson's Das. "I wanted to develop medicines to treat diseases. That is my focus. There are still many diseases with no cure."

And for those interested in doing this kind of research, there may not be a better time than the present. "I wish I was 20-30 years younger and getting into this business now," says Millennium's Bolen. "I look at the contributions that scientists in industry are making. The quality of science in biotech and pharma is world class and much is done in collaboration with academic labs. There is a high level of excitement in the scientific world."

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DOI: 10.1126/science.opms.r0900079